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9 green tablets containing 1.0 mg norethindrone with 0.035 mg ethinyl estradiol followed by
5 blue tablets containing 0.5 mg norethindrone with 0.035 mg ethinyl estradiol.
(7 orange tablets containing inert ingredients are included in the 28-day regimen.)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk. C—Use associated with moderate risk.
B—Use associated with high risk. D—Use associated with low risk

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

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GYN CME FUND SYMPOSIUM SCHEDULE FOR 1985

March 9-10

"Update in Diagnosis and Treatment of OB/GYN Infections"

Richard Schwarz, M.D. David Eschenbach, M.D. Gillis Monif, M.D.

June 22-23

"Medical & Surgical Problems of the Climacteric"

David H. Nichols, M.D. J. George Moore, M.D. Thomas T. Yoshikawa, M.D.

September 7-8

"Common Problems in Reproductive Endocrinology"

Jorge Mestman, M.D. Charles March, M.D. Sergio Stone, M.D. Allan DeCherney,

November 2-3

"Update in Maternal-Fetal Medicine"

Frank C. Miller, M.D. Jerry Wiley, J.D. Lawrence Platt, M.D. Julian P. Parer,

UPDATE IN DIAGNOSIS AND TREATMENT OF OB/GYN INFECTIONS

Saturday, March 9, 1985

7:30 AM Richard Schwarz, M.D.
"Selection of Antibiotics"
8:30 AM David Eschenbach, M.D.
"Chlamydial Infections"
9:30 AM Gillis Monif, M.D.
"Infections & Complications Associated with the IUD"
10:30 AM David Eschenbach, M.D.
"Vaginitis"
11:30 AM Gillis Monif, M.D.
"Changing Concepts in Acute Salpingitis"

Sunday, March 10, 1985

7:30 AM Richard Schwarz, M.D.
"Prematurity/An Infectious Disease?"
8:30 AM Gillis Monif, M.D.
"Chorioamnionitis: Diagnosis and Treatment"
9:30 A.M. David Eschenbach, M.D.
New Thoughts in the Pathogenesis and Bacteriology of Postpartum Hemorrhage"
10:30 AM Richard Schwarz, M.D.
"Group B Streptococcus in Pregnancy"
11:30 AM Gillis Monif, M.D.
"Hepatitis B in Pregnancy"

MEDICAL & SURGICAL PROBLEMS OF THE CLIMACTERIC

Saturday, June 22, 1985

7:30 AM David H. Nichols, M.D.
"The Pros and Cons of Estrogen Replacement"
8:30 AM Thomas T. Yoshikawa, M.D.
The Epidemiology and Demography of Aging and Its Impact on Clinical Practice"
9:30 AM David H. Nichols, M.D.
What the Gynecologist Should Know About Breast Disease in the Older Patient"
10:30 AM Thomas T. Yoshikawa, M.D.
"The Biological Changes and Medical Problems of Aging"
11:30 AM David H. Nichols, M.D.
"Oophorectomy in the Older Woman: Yes or No"

Sunday, June 23, 1985

7:30 AM Thomas T. Yoshikawa, M.D.
"Unique Problems of Urinary Tract Infection in the Aging Female"
8:30 AM David H. Nichols, M.D.
"The Diagnosis & Treatment of Urinary Incontinence"
9:30 AM J.G. Moore, M.D.
Pre & Post Operative Care in Elderly Patients Undergoing Vaginal Hysterectomy"
10:30 AM Thomas T. Yoshikawa, M.D.
"Special Considerations in use of Antibiotics in Elderly Patients"
11:30 AM J.G. Moore, M.D.
"Practical Features in the Technique of Vaginal Hysterectomy"

COMMON PROBLEMS IN REPRODUCTIVE ENDOCRINOLOGY

Saturday, September 7, 1985

7:30 AM Allen DeCherney, M.D.
"In Vitro Fertilization"
8:30 AM Charles March, M.D.
"What's New with GnRh"
9:30 AM Allen DeCherney, M.D.
"Ectopic Pregnancy"
10:30 AM Jorge Mestman, M.D.
"Evaluation and Management of Thyroid Disorders in Pregnancy"
11:30 AM Allen DeCherney, M.D.
"Infertility Surgery"

Sunday, September 8, 1985

7:30 AM Charles March, M.D.
"Current Status of Hysteroscopy in Gynecology"
8:30 AM Sergio Stone, M.D.
"The Evaluation and Work-up of the Patient with Excess Androgen Producti
9:30 AM Charles March, M.D.
"Current Status of Ultra Sound in Gynecology"
10:30 AM Sergio Stone, M.D.
"A Re-evaluation of the Cervical Factor in Infertility"
11:30 AM Jorge Mestman, M.D.
"Multiple Endocrine Deficiency Syndrome"

UPDATE IN MATERNAL-FETAL MEDICINE

Saturday, November 2, 1985

7:30 AM Julian P. Parer, M.D.
"Management of Abnormal Heart Rate Patterns During Pregnancy"
8:30 AM Frank C. Miller, M.D.
"Obstetrical Events Leading to Mentally Retarded Children"
9:30 AM Julian P. Parer, M.D.
"Update on Rh Disease"
10:30 AM Frank C. Miller, M.D.
"Management of Multiple Gestations"
11:30 AM Julian P. Parer, M.D.
"Four Ways to Decrease C/Section Rate"

Sunday, November 3, 1985

7:30 AM Lawrence Platt, M.D.
"New Methods of Fetal Assessment Including Biophysical Profile & Doppler Blood Flow Measurements"
8:30 AM Frank C. Miller, M.D.
"Antepartum & Intrapartum Management of Post-dated Pregnancies"
9:30 AM Lawrence Platt, M.D.
"Use of Ultrasound in Labor & Delivery"
10:30 AM Jerry Wiley, J.D.
"Is Suing Obstetricians the New National Pastime"
11:30 AM Lawrence Platt, M.D.
"Prenatal Diagnosis Update"

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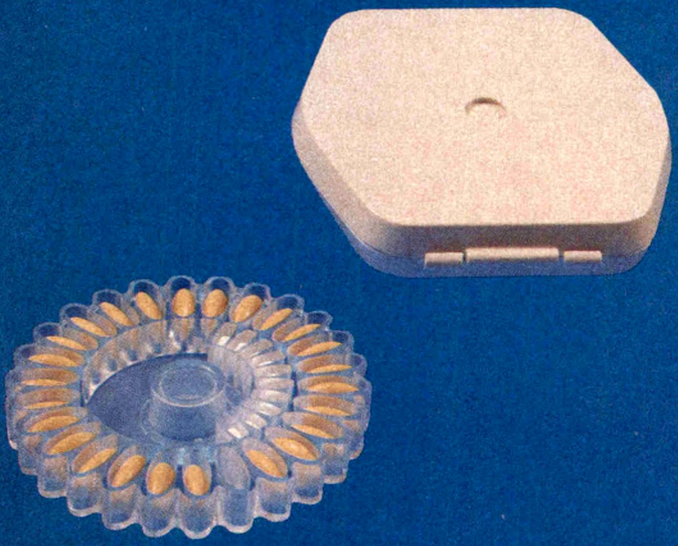

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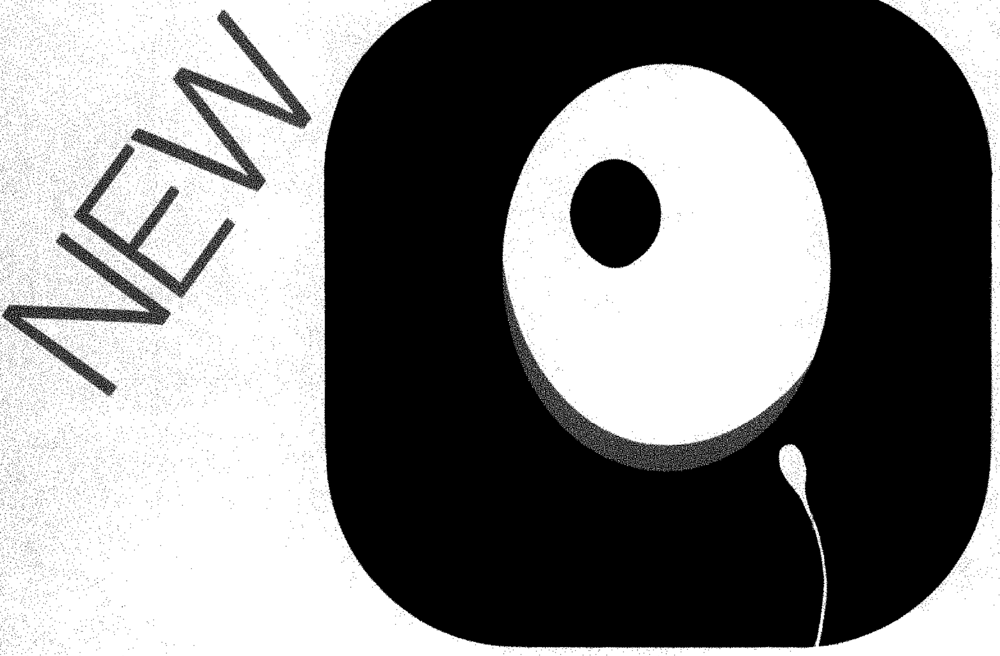

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[†] In infections due to susceptible strains of indicated organisms.

[‡] *In vitro* susceptibility does not necessarily imply a correlation with *in vivo* results.

[§] Based on PDR, 1984; Manufacturer's Prescribing Information.

[¶] In severe infections, or infections caused by less sensitive organisms, the total daily dose and/or frequency *may be* increased. Patients have been successfully treated with a total daily dosage of 6 to 12 grams divided into two, three, or four administrations ranging from 1.5 to 4 grams per dose.

[1] Carlin HS, Romankiewicz J: The impact of dosage regimen on intravenous drug cost. Scientific Exhibit, American Society of Hospital Pharmacists, New Orleans, Dec 6-10, 1981.

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PSEUDOMEMBRANOUS COLITIS HAS BEEN REPORTED WITH THE USE OF CEPHALOSPORINS (AND OTHER BROAD-SPECTRUM ANTIBIOTICS); THEREFORE, IT IS IMPORTANT TO CONSIDER ITS DIAGNOSIS IN PATIENTS WHO DEVELOP DIARRHEA IN ASSOCIATION WITH ANTIBIOTIC USE.

PRECAUTIONS: Although transient elevations of the BUN and serum creatinine have been observed, CEFOBID alone does not appear to cause significant nephrotoxicity. However, concomitant administration of aminoglycosides and other cephalosporins has caused nephrotoxicity.

CEFOBID is extensively excreted in bile. The serum half-life of CEFOBID is increased 2–4 fold in patients with hepatic disease and/or biliary obstruction. In general, total daily dosage above 4 g should not be necessary in such patients. If higher dosages are used, serum concentrations should be monitored.

Because renal excretion is not the main route of elimination of CEFOBID (see CLINICAL PHARMACOLOGY), patients with renal failure require no adjustment in dosage when usual doses are administered. When high doses of CEFOBID are used, concentrations of drug in the serum should be monitored periodically. If evidence of accumulation exists, dosage should be decreased accordingly.

The half-life of CEFOBID is reduced slightly during hemodialysis. Thus, dosing should be scheduled to follow a dialysis period. In patients with both hepatic dysfunction and significant renal disease, CEFOBID dosage should not exceed 1–2 g daily without close monitoring of serum concentrations.

As with other antibiotics, vitamin K deficiency has occurred rarely in patients treated with CEFOBID. Those at risk include patients with a poor nutritional status, malabsorption states (e.g., cystic fibrosis), alcoholism, and patients on prolonged hyper-alimentation regimens (administered either intravenously or via a naso-gastric tube). Prothrombin time should be monitored in these patients and exogenous vitamin K administered as indicated.

A disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia has been reported when alcohol (beer, wine) was ingested within 72 hours after CEFOBID administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOBID. A similar reaction has been reported with other cephalosporins.

Prolonged use of CEFOBID may result in the overgrowth of nonsusceptible organisms. Careful observation of the patient is essential.

CEFOBID should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Drug Laboratory Test Interactions: A false-positive reaction for glucose in the urine may occur with Benedict's or Fehling's solution.

Carcinogenesis, Mutagenesis, Impairment of Fertility: The maximum duration of CEFOBID animal toxicity studies is six months. In none of the *in vivo* or *in vitro* genetic toxicology studies did CEFOBID show any mutagenic potential at either the chromosomal or subchromosomal level. CEFOBID produced no impairment of fertility and had no effects on general reproductive performance or fetal development when administered subcutaneously at daily doses up to 500 to 1000 mg/kg prior to and during mating, and to pregnant female rats during gestation. These doses are 10 to 20 times the estimated usual single clinical dose.

Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in mice, rats, and monkeys at doses up to 10 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to CEFOBID. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Only low concentrations of CEFOBID are excreted in human milk. Although CEFOBID passes poorly into breast milk of nursing mothers, caution should be exercised when CEFOBID is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: In clinical studies the following adverse effects were observed and were considered to be related to CEFOBID therapy or uncertain etiology:

Hypersensitivity: As with all cephalosporins, hypersensitivity manifested by skin reactions (1 patient in 45), drug fever (1 in 260), or a change in Coombs' test (1 in 60) has been reported. These reactions are more likely to occur in patients with a history of allergies, particularly to penicillin.

Hematology: As with other beta-lactam antibiotics, reversible neutropenia may occur with prolonged administration. Slight decreases in neutrophil count (1 patient in 50) have been reported. Decreased hemoglobins (1 in 20) or hematocrits (1 in 20) have been reported, which is consistent with published literature on other cephalosporins. Transient eosinophilia has occurred in 1 patient in 10.

Hepatic: Of 1285 patients treated with cefoperazone in clinical trials one patient with a history of liver disease developed significantly elevated liver function enzymes during CEFOBID therapy. Clinical signs and symptoms of nonspecific hepatitis accompanied these increases. After CEFOBID therapy was discontinued, the patient's enzymes returned to pre-treatment levels and the symptomatology resolved. As with other antibiotics that achieve high bile levels, mild transient elevations of liver function enzymes have been observed in 5–10% of the patients receiving CEFOBID therapy. The relevance of these findings, which were not accompanied by overt signs or symptoms of hepatic dysfunction, has not been established.

Gastrointestinal: Diarrhea or loose stools has been reported in 1 in 30 patients. Most of these experiences have been mild or moderate in severity and self-limiting in nature. In all cases, these symptoms responded to symptomatic therapy or ceased when cefoperazone therapy was stopped. Nausea and vomiting have been reported rarely.

Symptoms of pseudomembranous colitis can appear during or for several weeks subsequent to antibiotic therapy (see WARNINGS).

Renal Function Tests: Transient elevations of the BUN (1 in 16) and serum creatinine (1 in 48) have been noted.

Local Reactions: CEFOBID is well tolerated following intramuscular administration. Occasionally, transient pain (1 in 140) may follow administration by this route. When CEFOBID is administered by intravenous infusion some patients may develop phlebitis (1 in 120) at the infusion site.

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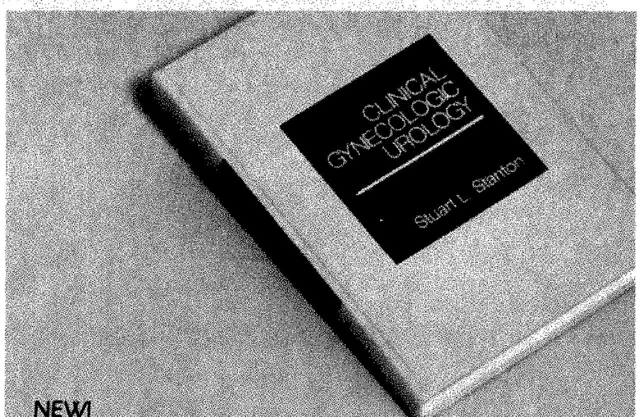

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"The fact that two lives are at risk, and the varying physiology during the course of pregnancy and post partum make the bravest of us need a source of help and instruction. The book edited by Michael de Swiet fulfills this role admirably . . . the book as a whole is excellent and highly recommended. It is a book to buy." (The Lancet)

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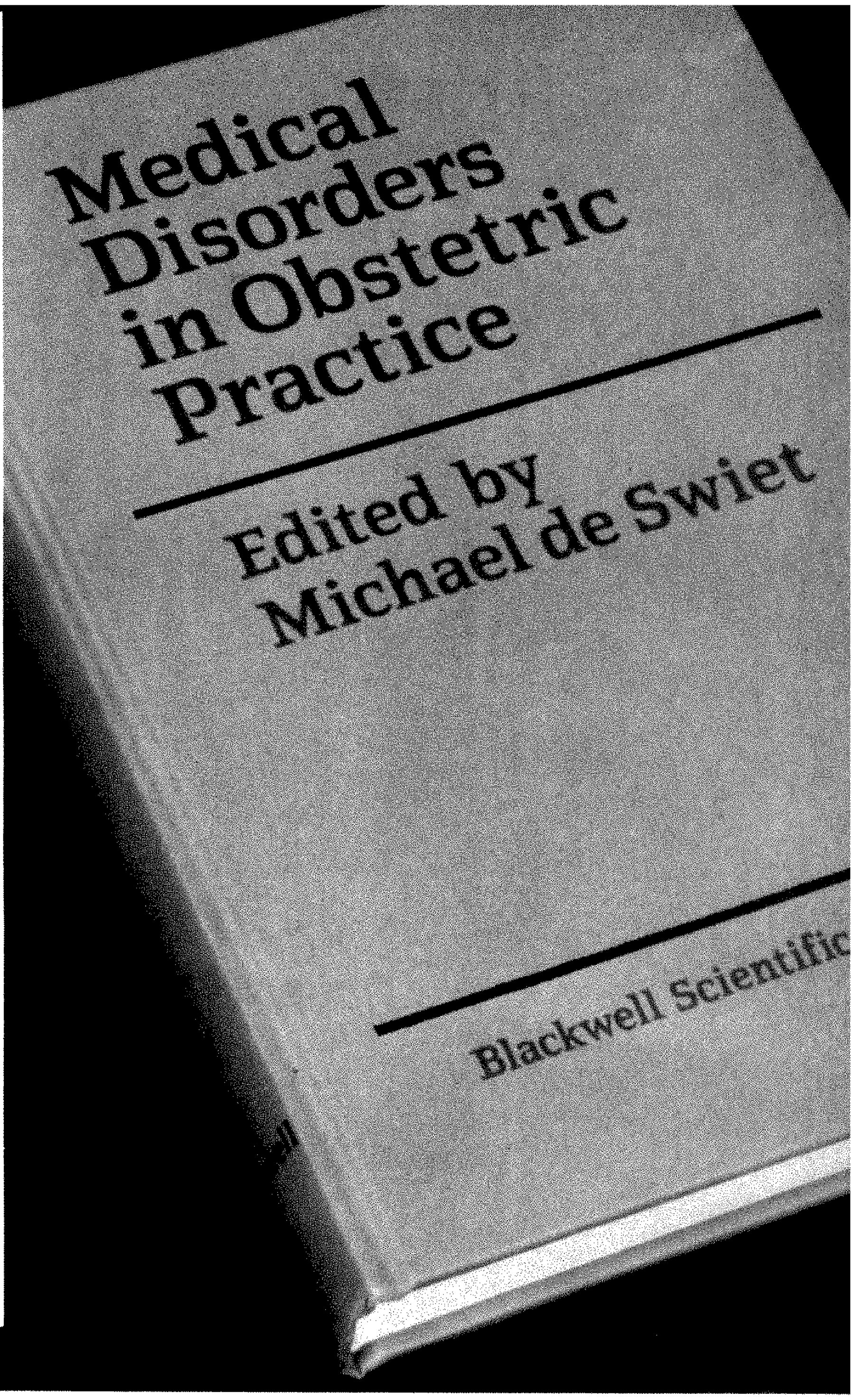
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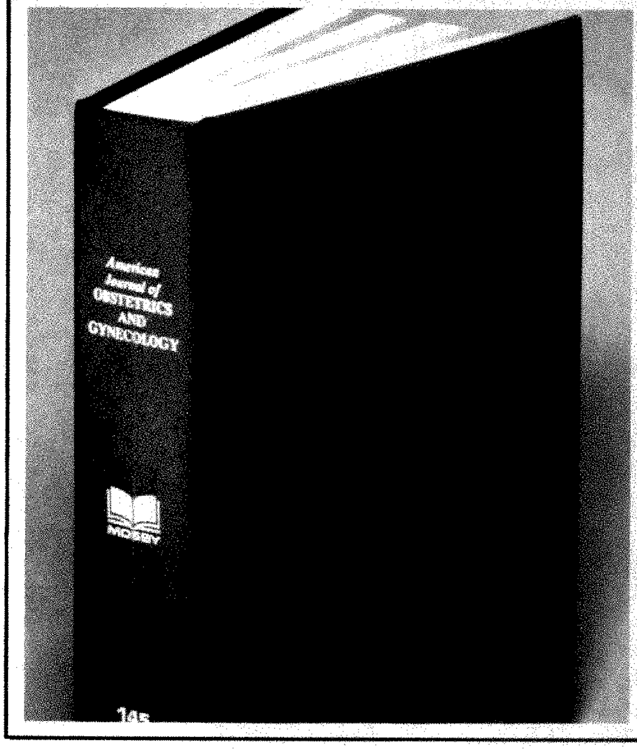

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1. Rössner S et al: *Acta Obstet Gynecol Scand* 59:255, 1980. 2. Nash AL et al: *Med J Aust* 2:277, 1979. 3. Larsson-Cohn U et al: *Fertil Steril* 35:172, 1981. 4. Briggs MH: *J Reprod Med* 28:92, 1983. 5. Åhrén T et al: *Contraception* 24:451, 1981. 6. Briggs MH, Briggs M: *Acta Obstet Gynecol Scand* Suppl 105:25, 1982.

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1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See Patient Package Labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See Warnings section #3, 4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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EDITORIAL

New format for the American Journal of Obstetrics and Gynecology

You will notice with this issue that a new format for the JOURNAL has been adopted. The clinical articles are grouped in one section of the JOURNAL and the basic science articles in another. The lead article in the Clinical Section will be a Clinical Opinion article to be followed by regular clinical articles, Brief Communications, Case Reports, Current Investigation articles, when available, and finally Clinical Current Development articles in some issues. The Basic Science section will have regular articles, Brief Communications, Current Investigation articles, and Current Development articles when available.

These changes are intended for the convenience of our readership. We realize some of the readers are primarily interested in clinical articles and others in basic science. A recent survey documented that many physicians choose articles by title alone; hence the new format will give easier access to their choices.

Some physicians have said they do not read the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY because it contains too much basic science. We are proud of the fact that many fine basic science investigators choose to publish their work in the JOURNAL. These articles represent twenty percent of all we publish. Our excellent clinical articles (as pointed out in the yearly statistics) outnumber basic science articles by four times, and we felt grouping the articles under a clinical heading might help convince the readers that the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY is a journal for all obstetricians and gynecologists. This was a mission established for the JOURNAL many years ago by the Editors.

The Editors and Publisher hope that you will like the new format.

The Editors

Autocorrelation techniques in fetal monitoring

Michael Y. Divon, M.D., Fred P. Torres, B.M.E., Sze-Ya Yeh, M.D., and Richard H. Paul, M.D.
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Fetal monitors that feature autocorrelation have recently been introduced. This paper discusses autocorrelation and describes how it extracts periodic signals from a noisy background. An example of its performance under clinical conditions demonstrating advantages and limitations is discussed. (Am J Obstet Gynecol 1985;151:2-6.)

Key words: Fetal heart rate, fetal monitoring, autocorrelation

Electronic fetal heart rate testing has become the standard of practice for antepartum assessment of fetal well-being. The most common method of external fetal heart rate monitoring utilizes Doppler ultrasound devices. These monitoring systems derive trigger signals from the reflection of sonic waves from the beating fetal heart. The fetal heart rate tracing is produced by measuring the intervals between successive trigger signals. This indirect, externally derived fetal heart rate is usually a reasonable representation of a true fetal heart rate. However, an absolute representation of a fetal heart rate can be obtained only from a direct electrode system.

During the last decade, a number of advances have been made in ultrasonic techniques. Recently, fetal monitors that feature autocorrelation in the ultrasound section of the instrument have been introduced. Autocorrelation as a method of signal processing is not new. It has long been used in communication systems to retrieve weak signals from noisy backgrounds. Its use in fetal monitoring was suggested by Favret in the early 60s.[1] Nevertheless, until the advent of the microprocessor, the implementation of this technique was commercially impractical.

The characteristics of any fetal monitoring system must be understood by the user, and particular attention must be paid to features that may induce artifact or lead to misinterpretation. All fetal monitors utilize signal processing and "logic" techniques to create an indirect fetal heart rate record. This indirect record will often differ from the "true," or actual, heart rate. The user must be fully aware of the effects of electronic manipulation and interpret the resultant heart rate within that context. It is the intent of this paper to discuss autocorrelation and to describe how it extracts periodic signals from a noisy background. An example of its performance under clinical conditions demonstrating advantages and limitations will be discussed.

Signal processing

The Doppler signal. The Doppler ultrasound signal is a periodic, bipolar signal immersed in noise. Arterial blood flow, fetal movements, maternal movements, or muscle contractions may randomly alter the received ultrasonic waves. The resulting composite signal (Fig. 1) consists of bursts of varying amplitudes and frequencies. These complex reflected signals cannot be easily associated with the motion of a specific cardiac structure. Likewise, signal characteristics are dramatically affected by transducer placement and movement. Until recently, to extract the relevant information, most manufacturers of fetal monitors have used an almost universal scheme that is best depicted by a block diagram of a conventional fetal monitor (Fig. 2). First, the incoming signal is amplified and demodulated to extract signals produced by moving anatomic structures. Filtering is then applied both at the low end (<200 Hz) and the high end (>600 Hz) to select and emphasize frequencies likely to be associated with fetal cardiac motions. The bipolar signal is converted to a unipolar

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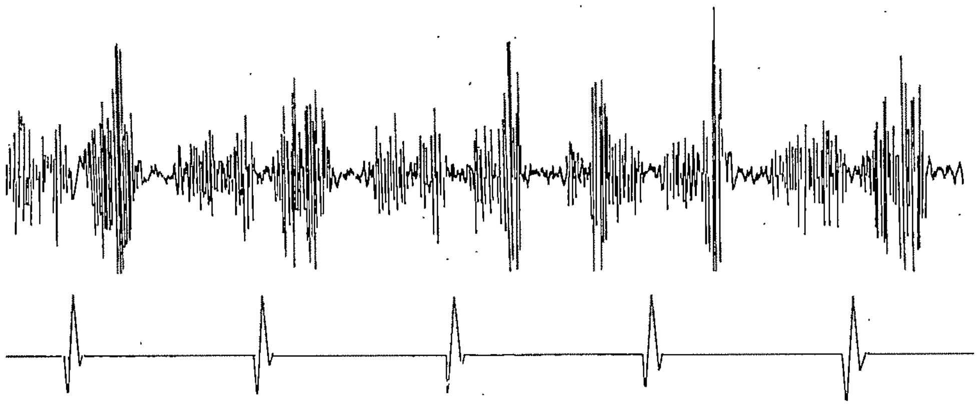

Fig. 1. Simultaneously recorded ultrasound and electrocardiogram signals.

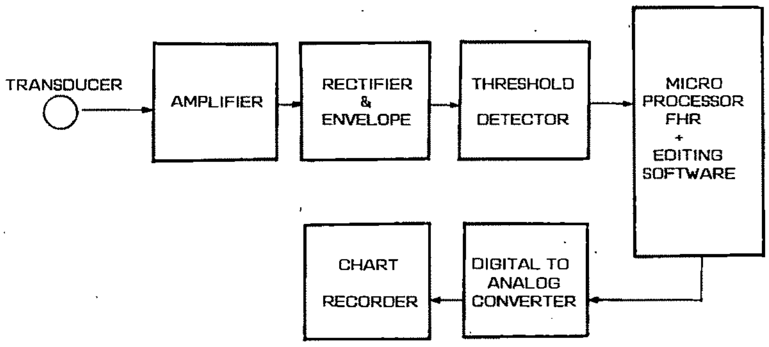

Fig. 2. Basic components of a conventional fetal monitor. *FHR,* Fetal heart rate.

one by electronic rectification. This is followed by further filtering to produce an output that indicates only the relative amplitude and timing of successive signal bursts. This signal is fed to a threshold or peak detector that produces a pulse for each significant burst. The pulse output of the peak detector is then fed to a microprocessor for additional processing.

Since no determination can be made of the specific anatomic origin of the Doppler signal bursts, consistency of timing between successive events is heavily relied on as a criterion to accept a pulse as valid or invalid. In an attempt to overcome the intrinsic problems due to the inconsistency of ultrasonic signals, software routines provide editing and "logic" to eliminate triggers that are likely to be artifacts. In addition, averaging over brief time periods is commonly used to "enhance" the appearance of the fetal heart rate pattern. Thus the fetal heart rate derived from these signals may under ideal circumstances closely approximate, but can never duplicate, the "true" fetal heart rate.

Autocorrelation. Autocorrelation has been used to detect weak signals immersed in random noise or to detect periodic signals from seemingly complex signal patterns.[2] In essence, it is a signal processing scheme that compares the incoming signal with a time-delayed version of itself. The useful information in the signal has a regular form that is repeated over time. At the same time the background noise has no such regularity. As a result, the useful information is augmented while the random noise is cancelled.

To understand how this method works, let us assume that two waveforms, A and B, are identical. If this fact was not known, a good method of measuring the similarity between A and B would be to multiply the waveforms, ordinate by ordinate, and then sum them over a period of time. For example, a_1, a_2, a_3, . . . a_n of A would be multiplied respectively by corresponding ordinates b_1, b_2, b_3, . . . b_n of B. These products are then summed to obtain a single value. The magnitude of this value is an expression of the degree of "sameness" between the two waveforms. If the waveforms are identical, every product ($a_n \times b_n$) is positive, even for negative ordinates, and the sum is a large positive number. If the waveforms are dissimilar, some ordinates are likely to be of opposite polarity and differing amplitude, and consequently the sum of the products will be a smaller number.

If we now take two identical waveforms (Fig. 3), but

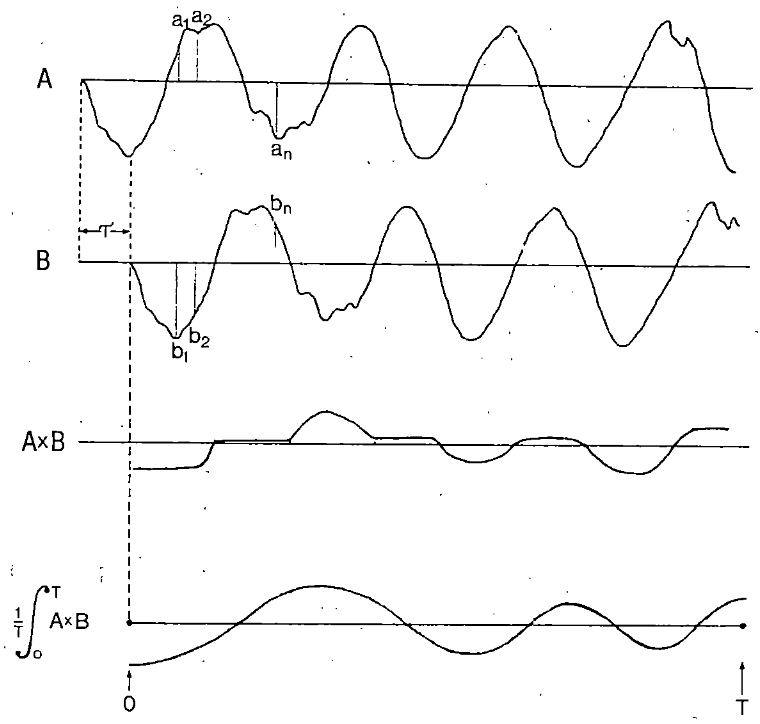

Fig. 3. Graphic computation of the autocorrelation function.

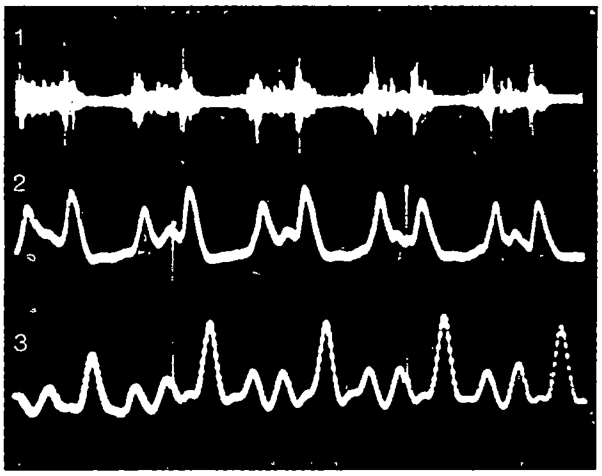

Fig. 4. A comparison between envelope and autocorrelation detection of an ultrasound signal. *1,* Ultrasound signal; *2,* its envelope; *3,* its autocorrelation function.

delay one, say B, by a time interval with respect to the other, the corresponding ordinates, a_n and b_n, are no longer identical. For a large delay, the ordinates may even assume different polarities. Thus positive products will tend to cancel negative products, and the resulting measure of "sameness" will be smaller. The process of comparing a waveform with itself as described above is termed autocorrelation.[3]

In a strict sense, autocorrelation can be performed only on a "stationary wave." For signals associated with ongoing processes, such as fetal heart rate signals, multiplication and summation can be continuous along the time axis as the waveforms are sampled at short time intervals. The computation takes place for each sampled data and the summation is made over a period designated as T. T is called the computation interval or time window and may extend over several cycles of the waveforms. The result is a new continuous time varying function called the autocorrelation function. This operation is described in the following mathematical form:

$$C = \frac{1}{T} \int_{0}^{T} f(t) \cdot f(t-\tau)\, dt$$

where C is the autocorrelation function, T the computation interval, f(t) the original waveform, τ the delay and f(t-τ) the delayed waveform.

A periodic signal will produce an autocorrelation function with the same period.[4] For a random signal such as noise (which has no constant or periodic components), the autocorrelation function approaches zero as the observation period increases. This is intuitively plausible since, given the random nature of noise and a large enough shift, the ordinates ($a_n \times b_n$) will not increase in amplitude as is the case with periodic functions, but rather will tend to cancel out. Thus a periodic waveform with superimposed noise will produce a periodic autocorrelation function with very little noise superimposed on it. Further mathematical discussion of noise and its autocorrelation function is beyond the scope of this paper. A formal presentation of this subject can be found in a book by Lee.[5]

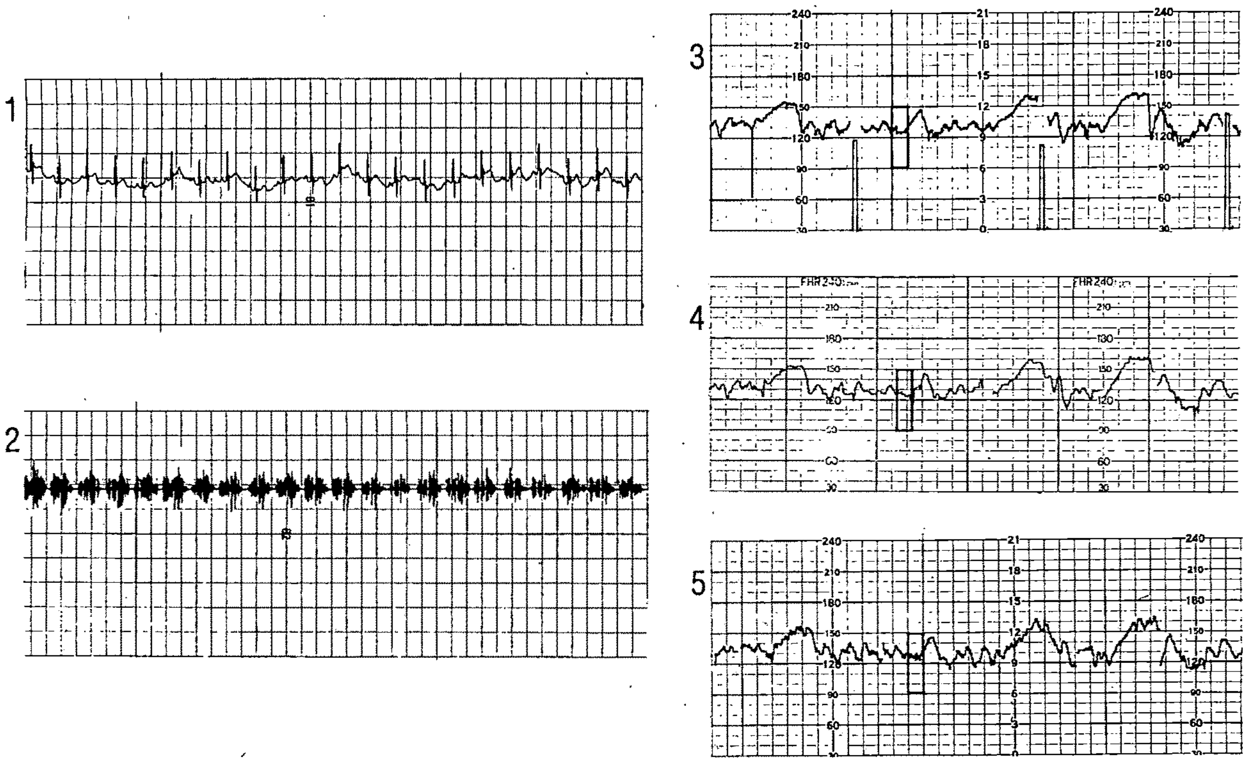

Fig. 5. Original fetal electrocardiogram and simultaneously recorded clean ultrasound signals. *1,* Electrocardiogram; *2,* ultrasound; *3,* direct fetal heart rate; *4,* "autocorrelation" fetal heart rate; *5,* "conventional" fetal heart rate. Heart rate in boxed areas is derived from the original signals shown at left.

The ability of autocorrelation to enhance the periodicity present in a signal and at the same time to decrease the contribution caused by random noise makes it a potentially beneficial method for processing ultrasound signals in a fetal monitor.

Fig. 4 shows the original ultrasonic signal and the envelope obtained by a conventional fetal monitor after filtering and rectification. Such a waveform would be difficult to "count," since the threshold detector would have to discriminate between peaks of virtually the same magnitude. The resultant count would, in all probability, be out of range or would have a large variable component. As a contrast, the autocorrelation function derived from the same ultrasonic wave features peaks that are easy to count and the ensuing variability will be low (Fig. 4).

Recently we have examined the accuracy of abdominally recorded ultrasound fetal heart rate signals relative to those obtained via direct fetal electrocardiogram. Fetal heart activity was simultaneously recorded during labor by means of a scalp electrocardiogram (Corometrics Fetal Monitor 112), and an external ultrasound detector that incorporates autocorrelation in its design (Corometrics Fetal Monitor 115). In order to assess the effect of autocorrelation on the ultrasound signal, the raw signal was recorded (Database CPR 4010B) and then replayed into the ultrasound section of a conventional monitor (i.e., one that does not use autocorrelation techniques) (Corometrics Fetal Monitor 112).

Recent experience with these systems has provided observations that emphasize the ability of the system that uses autocorrelation to extract the ultrasound signal from a very noisy background. The direct fetal heart rate, which was derived via a fetal scalp electrode, is presented in Fig. 5. This is the absolute representation of the fetal heart rate pattern, and the standard against which other techniques must be judged. The ultrasonic signal for the same period of time is also presented in Fig. 5. As seen, the signal-to-noise ratio is high and both monitors (i.e., with and without autocorrelation) did provide a reasonable reproduction of the actual fetal heart rate. On the other hand, when the signal-to-noise ratio was low, as in Fig. 6, the autocorrelation-based ultrasound system did fairly well, whereas the conventional design could not produce an interpretable fetal heart rate tracing. These fetal heart rate tracings are compared to the simultaneously recorded direct fetal heart rate in Fig. 6.

Comment

An externally recorded fetal heart rate should not be considered to be as accurate as a direct fetal heart rate, irrespective of the technique used to provide the

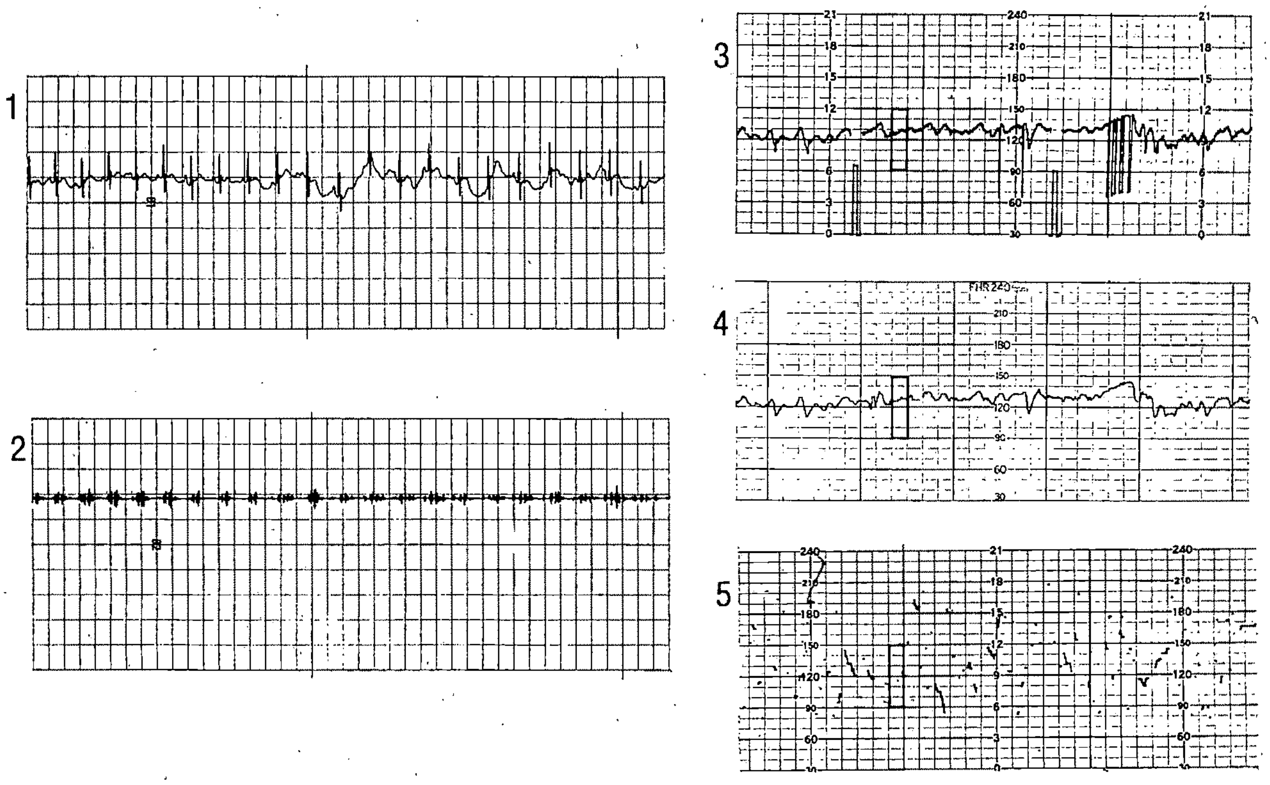

Fig. 6. Original fetal electrocardiogram and simultaneously recorded noisy ultrasound signals. *1,* Electrocardiogram; *2,* ultrasound; *3,* direct fetal heart rate; *4,* "autocorrelation" fetal heart rate; *5,* "conventional" fetal heart rate. Heart rate in boxed areas is derived from the original signal shown at left.

external tracing. A recent report by Amato[6] provides documentation of the errors inherent to an indirect, ultrasonically derived fetal heart rate and its potential for misinterpretation. Lawson et al.[7] compared an autocorrelation system to a direct fetal electrocardiogram and to a conventional external monitor. They concluded that a reduction in signal loss has been achieved relative to the nonautocorrelation system and that the accuracy of the autocorrelation system was "within acceptable limits" relative to the direct electrocardiogram.

Autocorrelation enhances signal-to-noise levels of periodic phenomena and helps in detecting fetal heart rate from a noisy background. In the absence of fetal heart signals, it may produce a factitious "heart rate" by detecting any periodic motion originating from the mother and/or the fetus (maternal heart rate, fetal breathing movements, etc.). It is therefore important to realize that whenever there is doubt as to the origin of the signals that are displayed as a fetal heart rate or whenever clinical action is to be taken based on the externally derived fetal heart rate, the validity of these observations should be verified. Auscultation or direct electrocardiogram could be used for this purpose.

With the clinical application of new techniques, unexpected findings are likely to surface. In order to avoid incorrect interpretation or intervention, caution must be advised. Vigilance and an intelligent approach to unusual occurrences will lead to definitive observations. It is hoped that this article will assist care providers with an organized and thoughtful introduction of a new monitoring technique.

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The significance of a previous stillbirth

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Among 7052 patients studied between 1976 and 1982 in a collaborative project on antepartum fetal heart rate monitoring, 337 patients had a previous stillbirth as a reason for testing. Overall a previous stillbirth history significantly increased the risk of having a positive result on a contraction stress test, primarily among hypertensive patients. Patients with a previous stillbirth also had a significantly higher incidence of respiratory distress syndrome in their neonates attributable to premature intervention for maternal indications (primarily among hypertensive women and patients with clinical intrauterine growth retardation). Low Apgar scores were found to be significantly increased in diabetics with previous stillbirths primarily due to neonates with congenital malformations. Premature intervention by labor induction or cesarean section was more common among patients with a previous stillbirth for both maternal indications and abnormal antepartum fetal heart rate studies. Previous stillbirth would appear therefore to be a significant risk factor primarily when associated with a diagnosis of hypertension or clinical intrauterine growth retardation. (AM J OBSTET GYNECOL 1985;151:7-13.)

Key words: Stillbirth, contraction stress test, morbidity, mortality

A history of a previous stillbirth is a well-accepted risk factor for increased mortality in a subsequent pregnancy. Most classifications regard a previous stillbirth history as among the most significant of all historic risk factors.[1-10] The Collaborative Perinatal Study conducted by the National Institute of Neurological Diseases and Stroke showed that patients whose previous pregnancy had resulted in a stillbirth had a perinatal mortality of 73 per 1000 in the next pregnancy and 1.7% of their surviving offspring were neurologically abnormal at 1 year.[9] The 1963 British perinatal mortality study showed that a history of a previous perinatal death more than doubled the subsequent risk of pregnancy loss and/or premature delivery histories.[10] The data from these two historic studies were gathered prior to modern methods of antepartum fetal surveillance techniques and therefore prior to an era of pregnancy intervention for fetal reasons.

This study represents an attempt to evaluate the significance of a history of previous stillbirth among a group of patients followed with antepartum fetal heart rate testing.

Material and methods

The study group consisted of 308 patients with a history of previous stillbirth among 7052 high-risk patients undergoing antepartum fetal heart rate testing. The data for this study were gathered from 19 institutions participating in a collaborative study on antepartum fetal heart rate testing between 1976 and 1980, the details of which were previously reported.[10-12]

An additional 29 Long Beach Women's Hospital patients with previous stillbirth subsequently added between 1980 and 1982 bring the number of patients with previous stillbirth to 337. Each patient's clinical data as well as specific data on each antepartum test were prospectively coded for subsequent computer analysis.

Excluded from the 7448 patients presented previously[11, 12] were 396 patients whose testing was not satisfactory or who were not compliant. This included three patients with fetal deaths who were excluded because they were not satisfactorily tested. Their histories are as follows:

Case 1. The patient was a Class B diabetic with a previous stillbirth who had a reactive negative contraction stress test at 36 weeks' gestation but was not retested for 18 days. She reappeared in labor at 39 weeks' gestation and was delivered of a 2810 gm stillborn infant with a tight nuchal cord.

Case 2. The patient was a Class A diabetic with a

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Table I. Diagnoses of study patients*

Diagnosis	*No. of patients*
Total of patients studied	7052
Patients with previous stillbirth	337
Previous stillbirth only	163
Previous stillbirth and hypertension	83
Previous stillbirth and diabetes	71
Previous stillbirth and clinical intrauterine growth retardation	29
Previous stillbirth and postdates	21

*More than one additional diagnosis possible.

previous stillbirth and massive obesity. She had one test that was completely unsatisfactory for technical reasons at 36 weeks' gestation and 7 days later was delivered of a 5840 gm stillborn infant with multiple anomalies.

Case 3. The patient was a Class A diabetic with a previous stillbirth who had one unsatisfactory test at 35 weeks' gestation and 2 weeks later was delivered of a 4280 gm stillborn infant with a "cord accident."

One hundred sixty-three of the 337 patients with a previous stillbirth had no other reason for testing. Of the 174 remaining patients with at least one other reason for testing, 71 were diabetic, 83 were hypertensive, 29 had clinically diagnosed intrauterine growth retardation, and 21 were postdate. (Table I). The average number of antepartum fetal heart rate tests per patient with a previous stillbirth was 5.0 compared to 2.5 tests per patient for the total population studied.

Antepartum testing results

Two hundred eight of the total 7052 patients studied had positive contraction stress test results (3.0%). Nineteen of the 337 patients with a previous stillbirth had positive contraction stress test results (5.6%) ($p < 0.05$). This difference was primarily accounted for by the patients with previous stillbirth plus hypertension (12.0% positive contraction stress test) and patients with previous stillbirth plus a clinical diagnosis of intrauterine growth retardation (17.2% positive contraction stress test). Patients with a previous stillbirth as the only reason for testing could not be shown to have a higher risk of having a positive contraction stress test (3.7%) than all tested patients (3.0%) (Table II).

Among patients with hypertension, those with a previous stillbirth had a greater risk of having a positive contraction stress test (12.0%) than did hypertensive patients with no previous stillbirth (4.3%) ($p < 0.01$) (Table III). Among patients with diabetes a previous stillbirth history could not be shown to increase the risk of a positive contraction stress test, but there was a trend in that direction (5.6% versus 3.6%) (Table III). Although 17.2% of patients with clinical intrauterine growth retardation and previous stillbirth had positive contraction stress test results compared to 8.2% of those patients with clinically diagnosed intrauterine growth retardation and no previous stillbirth, this difference was not statistically significant (Table III). There was also no increased incidence of positive contraction stress test results among postdate patients with previous stillbirth than among those patients that were without a previous stillbirth (Table III).

Table II. Antepartum fetal heart rate testing results and incidence of a positive contraction stress test

	No.	*Positive contraction stress test*	*%*
All patients studied (1976-1980)	7052	208	3.0
Patients without previous stillbirth (1976-1980)	6744	194	2.9
Patients with previous stillbirth (1976-1982)	337	19	5.6*
Previous stillbirth and hypertension	83	10	12.0*
Previous stillbirth and diabetes	71	4	5.6
Previous stillbirth and clinical intrauterine growth retardation	29	5	17.2*
Previous stillbirth and postdates	21	0	—
Previous stillbirth as only reason for antepartum fetal heart rate testing	163	6	3.7

*Statistically significant ($p < 0.05$) when compared by χ^2 to patients without previous stillbirth.

Perinatal outcome results

Patients with a previous stillbirth did not have a higher incidence of growth retardation, low 5-minute Apgar scores, late deceleration in labor, or perinatal deaths than the general population of patients studied (Table IV). There was, however, a significantly higher incidence of respiratory distress syndrome in patients with a previous stillbirth (3.9%) than in all other tested patients (1.7%) ($p < 0.05$).

Table V compares outcome parameters (congenital anomalies, neonatal weight below the tenth percentile, 5-minute Apgar scores, late deceleration in labor, stillbirth, neonatal death, and respiratory distress syndrome) between patients with a sole diagnosis of previous stillbirth and those with a diagnosis of previous stillbirth plus diabetes, hypertension, clinical IUGR, and late dates. Congenital anomalies were highest among diabetics (7.0%), but no statistically significant differences were demonstrated. There also were no statistical differences between groups in occurrence of late deceleration in labor, stillbirth, and neonatal death. Although only 31% of those with a clinical diagnosis of intrauterine growth retardation actually delivered

Table III. Relationship of hypertension, diabetes, clinical intrauterine growth retardation, postdates, and history of previous stillbirth to incidence of positive contraction stress test

	Previous stillbirth			*No previous stillbirth*			
Diagnosis	*No.*	*Positive contraction stress test*	*%*	*No.*	*Positive contraction stress test*	*%*	*p**
Hypertension							
Chronic hypertension	46	7	15.2	796	40	5.0	<0.01
Preeclampsia	32	3	9.3	1031	42	4.2	NS
Other hypertension	5	0	—	149	2	1.3	NS
Hypertension total	83	10	12.0	1976	84	4.3	<0.01
Diabetes							
Class A	21	1	4.8	514	13	2.5	NS
Class B-R	50	3	6.0	394	20	5.1	NS
Diabetes total	71	4	5.6	908	33	3.6	NS
Clinical intrauterine growth retardation	29	5	17.2	658	54	8.2	NS
Postdates	21	0	0	3092	51	1.6	NS

*Statistical comparisons by χ^2 or Fisher's exact test depending on cell size.

neonates with birth weight below the tenth percentile for gestational age, this was significantly higher than for patients without a clinical diagnosis of IUGR ($p < 0.01$). The incidence of 5-minute Apgar scores below 7 was significantly higher only among diabetics with previous stillbirth (8.5%) ($p < 0.05$). This was due to neonates with congenital malformations. The occurrence of respiratory distress syndrome was significantly more common among hypertensive patients with a previous stillbirth (9.6%) ($p < 0.01$) and patients with a previous stillbirth and clinical intrauterine growth retardation (10.3%) ($p < 0.01$) than among those patients with only a history of previous stillbirth (0.6%).

Among patients with hypertension the only outcome parameter that differed significantly between those with a previous stillbirth (9.6%) and those without a previous stillbirth (3.0%) was the incidence of respiratory distress syndrome ($p < 0.05$) (Table VI). Among diabetics the only outcome parameter that differed significantly between those with a previous stillbirth (8.5%) and those without a previous stillbirth (3.0%) was the 5-minute Apgar score below 7 ($p < 0.05$) (Table VII). Among patients with clinically diagnosed intrauterine growth retardation the only significant outcome parameter difference was for a neonatal weight percentile below 10 (31.0% for those with a previous stillbirth versus 10.5% for those with no previous stillbirth) ($p < 0.01$) (Table VIII). Table IX shows that no outcome parameters differed between postdate patients with a previous stillbirth and those with none.

Table IV. Perinatal outcome for patients with and without a previous stillbirth

	Previous stillbirth (n = 337)		*All other tested patients without previous stillbirth (n = 6744)*		
Outcome	*No.*	*%*	*No.*	*%*	*p**
Weight percentile ≤10	17	5.0	368	5.5	NS
5 min apgar score <7	13	3.9	178	2.6	NS
Late deceleration	29	8.6	664	9.8	NS
Fetal death	0	—	25	0.4	NS
Neonatal death	1	0.3	52	0.8	NS
Respiratory distress syndrome	13	3.9	112	1.7	<0.05

*Statistical comparisons by χ^2 or Fisher's exact test depending on cell size.

Intervention results

Among patients with a diagnosis of a previous stillbirth only 39.3% had pregnancy terminations before spontaneous labor began compared to 72.4% of diabetics with previous stillbirth ($p < 0.05$), 66.3% of hypertensive patients with a previous stillbirth ($p < 0.05$), and 65.5% of patients with clinical intrauterine growth retardation plus a previous stillbirth ($p < 0.05$). Only diabetics with a previous stillbirth had significantly more induced labor (36.6%) than patients with previous stillbirth alone (19.6%) ($p < 0.05$) (Table X).

Maternal indication for intervention was significantly higher among patients with a previous stillbirth and with hypertension (19.3%) ($p < 0.01$), those with diabetes (16.9%) ($p < 0.01$), and those with clinical intrauterine growth retardation (20.7%) ($p < 0.05$) than in patients with previous stillbirth only (5.5%). Intervention because of abnormal antepartum fetal heart rate testing results were significantly higher among hypertensive women with a previous stillbirth (19.3%) ($p < 0.01$) and patients with clinical intrauterine growth re-

Table V. Previous stillbirth and perinatal outcome

Diagnosis	No. of patients	*Congenital anomaly*		*Weight percentile <10*		*5 min Apgar score <7*		*Late deceleration in labor*		*Stillbirth*		*Neonatal death*		*Respiratory distress syndrome*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Previous stillbirth only	163	4	2.5	7	4.3	3	1.8	15	9.2	0	0	0	0	1	0.6
Previous stillbirth and diabetes	71	5	7.0	0	0	6	8.5*	6	8.5	0	0	0	0	4	5.6
Previous stillbirth and hypertension	83	2	2.4	10	12.0	4	4.8	8	9.6	0	0	1	1.2	8	9.6†
Previous stillbirth and clinical intrauterine growth retardation	29	1	3.4	9	31.0†	2	6.9	3	10.3	0	0	1	3.4	3	10.3*
Previous stillbirth and postdates	21	0	0	0	0	1	4.8	2	9.5	0	0	0	0	0	0

Statistical comparisons by χ^2 or Fisher's exact test depending on cell size.
*p < 0.05 when compared to patient with previous stillbirth only.
†p < 0.01 when compared to patients with previous stillbirth only.

Table VI. Perinatal outcome for patients with hypertension and previous stillbirth compared to those with hypertension and no previous stillbirth

Outcome	*Previous stillbirth plus hypertension (n = 83)*		*No previous stillbirth plus hypertension (n = 1976)*		*p**
	No.	*%*	*No.*	*%*	
Weight percentile <10	10	12.0	152	7.7	NS
5 min Apgar score <7	4	4.8	77	3.9	NS
Late deceleration	8	9.6	186	9.4	NS
Stillbirth	0	—	13	0.7	—
Neonatal death	1	1.2	20	1.0	—
Respiratory distress syndrome	8	9.6	60	3.0	<0.05

*Statistical comparisons by the χ^2 or Fisher's exact test depending on cell size.

Table VII. Perinatal outcome for diabetics with previous stillbirth compared to diabetics without previous stillbirth

Outcome	*Previous stillbirth plus diabetes (n = 71)*		*No previous stillbirth plus diabetes (n = 908)*		*p**
	No.	*%*	*No.*	*%*	
Weight percentile <10	0	—	26	2.9	NS
5 min Apgar score <7	6	8.5	27	3.0	<0.01
Late deceleration	6	8.5	70	7.7	NS
Stillbirth	0	—	4	0.4	NS
Neonatal death	0	—	10	1.1	NS
Respiratory distress syndrome	4	5.6	25	2.8	NS

*Statistical comparisons by χ^2 or Fisher's exact test depending on cell size.

tardation plus a previous stillbirth (27.6%) (p < 0.01) compared to patients with previous stillbirth only (7.4%) (Table XI).

Comment

There is general agreement among obstetricians that history of a previous stillbirth is a significant risk factor for poor subsequent-pregnancy outcome. Among the various risk scoring systems where a weighted risk value is arbitrarily assigned, a previous stillbirth history usually carries the maximum assigned weight. If we extrapolate from the collaborative study data[9] in which perinatal mortality in the pregnancy following a stillbirth was 7.3%, we would have expected 25 perinatal deaths among the 337 patients with a previous stillbirth in this study. Actually only four perinatal deaths occurred, but in patients satisfactorily studied with antepartum fetal heart rate testing there were no stillbirths and only one neonatal death. Certainly many things have changed in the past 25 years to account for the improved outcome but it would appear that antepartum fetal heart rate testing offers significant protection to patients with a previous stillbirth history. Since perinatal death is now rare even among high-risk patients followed with antepartum fetal heart rate testing, we must turn to these test results, morbidity out-

Table VIII. Perinatal outcome for patients with clinical intrauterine growth retardation and previous stillbirth compared to those with clinical intrauterine growth retardation with no previous stillbirth

	Previous stillbirth plus clinical intrauterine growth retardation (n = 29)		*No previous stillbirth plus clinical intrauterine growth retardation (n = 658)*		
Outcome	*No.*	*%*	*No.*	*%*	*p**
Weight percentile <10	9	31.0	69	10.5	<0.01
5 min Apgar score <7	2	6.9	31	4.7	NS
Late deceleration	3	10.3	81	12.3	NS
Stillbirth	0	—	5	0.8	NS
Neonatal death	1	3.4	15	2.3	NS
Respiratory distress syndrome	3	10.3	26	4.0	NS

*Statistical comparisons by χ^2 or Fisher's exact test depending on cell size.

Table IX. Perinatal outcome for patients with postdates and previous stillbirth compared to those with postdates and no previous stillbirth

	Previous stillbirth plus postdates (n = 21)		*No previous stillbirth plus postdates (n = 3092)*		
Outcome	*No.*	*%*	*No.*	*%*	*p**
Weight percentile <10	0	—	51	1.6	NS
5 min Apgar score <7	1	4.8	76	2.5	NS
Late deceleration	2	9.5	341	11.0	NS
Stillbirth	0	—	8	0.3	NS
Neonatal death	0	—	13	0.4	NS
Respiratory distress syndrome	0	—	10	0.3	NS

*Statistical comparisons by Fisher's exact test.

come parameters, and need for intervention to evaluate the impact of risk factors today.

In an attempt to measure the significance of a previous stillbirth as an independent variable, we found that a history of previous stillbirth was significantly correlated with a higher risk of having a positive contraction stress test and neonatal respiratory distress syndrome.

The group at least risk for a positive contraction stress test in a collaborative antepartum fetal heart rate testing study were those tested because of postdates (1.6%).[10] Because the largest percentage of patients in the total collaborative antepartum fetal heart rate testing study were tested because of late dates (44.3%)[11] and the previous stillbirth subgroup had only 6.2% late dates, the increased risk of a positive contraction stress test in patients with a previous stillbirth could have been accounted for solely because previous stillbirth is more common among hypertensive women, diabetics, and clinically diagnosed intrauterine growth retardation patients, and history of previous stillbirth itself may not actually influence test results. It was therefore decided to look at the risk of previous stillbirth with no other diagnosis. It was found that this group could not be shown to have a higher incidence of positive contraction stress test results (3.7% versus 3.0%) than the total population tested. Since it thus appeared that at least part of the increased incidence of positive contraction stress tests among the total previous stillbirth subgroup was linked to a higher incidence of hypertension and clinical intrauterine growth retardation associated with previous stillbirth, it was decided to study the influence of previous stillbirth within those groups of patients with diagnoses of hypertension, diabetes, clinical intrauterine growth retardation, and postdates.

In this study it was found that a history of previous stillbirth significantly increased the risk for a positive contraction stress test among hypertensive (predominantly chronic hypertensive) patients, and there was a strong but not significant trend among patients with clinical intrauterine growth retardation. Previous stillbirth could not be shown to influence risk for a positive contraction stress test among diabetics or postdate patients.

With regard to neonatal outcome, history of previous stillbirth increased the risk for respiratory distress syndrome only in hypertensive patients, low 5-minute Apgar scores only in diabetics, and small-for-gestational age neonates only among patients with clinical intrauterine growth retardation as a reason for testing.

Intervention for delivery was significantly more common when history of previous stillbirth was associated with diabetes, hypertension, or clinical intrauterine growth retardation than when previous stillbirth was a sole diagnosis or when it was associated with postdate pregnancy.

Indications for intervention influencing this difference were maternal reasons as well as abnormal antepartum fetal heart rate testing among patients with hypertension and those with clinical intrauterine growth retardation and only maternal reasons in diabetics. The higher incidence of respiratory distress syndrome in hypertensive patients and in those patients with clinical intrauterine growth retardation was probably due to the fact that intervention was more commonly necessary prior to fetal maturity in these groups.

Table X. Obstetric intervention in cases of previous stillbirth

Diagnosis	*No. of patients*	*Spontaneous labor*		*Induced labor*		*Cesarean section—no labor*		*Primary cesarean section—no labor*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Previous stillbirth only	163	99	60.7	36	19.6	32	19.6	13	8.0
Previous stillbirth and diabetes	71	19	27.6*	26	36.6*	26	36.6*	13	18.3*
Previous stillbirth and hypertension	83	28	33.7*	22	26.5	33	39.8*	20	24.1*
Previous stillbirth and clinical intrauterine growth retardation	29	10	34.5*	4	13.8	15	51.7*	10	34.5*
Previous stillbirth and postdates	21	13	61.9	8	38.1	0	0	0	0

*$p < 0.05$ compared to previous stillbirth only (χ^2).

Table XI. Reasons for obstetric intervention in cases of previous stillbirth

Diagnosis	*No. of patients*	*Abnormal antepartum fetal heart rate testing*		*Abnormal estriol*		*Maternal reasons*	
		No.	*%*	*No.*	*%*	*No.*	*%*
Previous stillbirth only	163	12	7.4	6	3.7	9	5.5
Previous stillbirth and diabetes	71	9	12.7	4	5.6	12	16.9*
Previous stillbirth and hypertension	83	16	19.3*	9	10.8	16	19.3*
Previous stillbirth and clinical intrauterine growth retardation	29	8	27.6*	4	13.8	6	20.7†
Previous stillbirth and postdates	21	3	14.2	2	9.5	0	0

Statistical comparisons by χ^2 or Fisher's exact test depending on cell size.
*$p < 0.01$ when compared with patients with previous stillbirth only.
†$p < 0.05$ when compared with patients with previous stillbirth only.

These data suggest therefore that a history of previous stillbirth does have an influence on contraction stress test results and outcome parameters, but it is primarily seen within a group of patients with hypertension, clinical intrauterine growth retardation, or both. Certainly, however, as an isolated diagnosis, history of previous stillbirth carries a significantly higher risk for a positive contraction stress test than does postdate pregnancy (3.7% versus 1.6%) ($p < 0.05$) and therefore would continue to be an indication for antepartum fetal heart rate testing.

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Post–tubal sterilization syndrome—A misnomer

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Menstrual parameters and gynecologic symptoms of 389 women who underwent laparoscopic tubal banding were studied prospectively and longitudinally. Previous oral contraceptive users exhibited an immediate increase in menstrual flow and dysmenorrhea, which declined slightly with time. No such changes occurred in women who used other methods of temporary contraception. A significant increase in noncyclic pelvic pain, independent of previous contraceptive usage, developed only in women >38 years of age. Menstrual cycles and dyspareunia were not affected. A small control group of women whose husbands underwent vasectomy showed no significant changes in any of these parameters. (AM J OBSTET GYNECOL 1985;151:13-9.)

Key words: Prospective, longitudinal, vasectomy control group, menstrual flow and pelvic pain, previous contraceptive usage

Aberrations of menstrual function, specifically, menorrhagia and/or pelvic pain, have been reported to occur following tubal sterilization.[1-6] Studies have demonstrated luteal phase deficiencies to account for the menorrhagia.[7, 8] Many gynecologists with wide experience in tubal sterilization have not observed these phenomena in their patients and have doubted the existence of adverse sequelae resulting from tubal sterilization.[9] Part of the controversy rests on the absence of a large prospective longitudinal study that analyzes a single technique, quantifies the alleged symptoms, takes into account preexisting pelvic pathologic conditions, and controls for the prior use and relinquishment of oral contraceptives and intrauterine contraceptive devices.

Most previous studies were small and retrospective. Only the study of Neil et al.[3] was controlled, but it did not accurately assess the role of relinquishing the use of oral contraceptives or intrauterine devices. One large, multicenter, multinational study which refuted the existence of adverse sequelae was longitudinal, but assessment of menstrual flow was subjective, follow-up rate was quite low, and a variety of techniques were considered together.[10] Another, more recent multicenter study also cast doubt on the development of significant menstrual changes following several types of tubal sterilization.[11] There were two serious problems in the design of that study. Patients were asked to recall the characteristics of their "natural" cycles while not using oral contraceptives or an intrauterine contraceptive device, regardless of how much time had passed since they had natural cycles. The second flaw, which the authors addressed and discounted, was that they excluded the 2.7% of patients who had hysterectomies, mostly for bleeding, during the follow-up period. Neither of the multicenter studies analyzed noncyclic pelvic pain.

The purpose of our study was to overcome the problems enumerated above and to answer the question, Is there a post–tubal sterilization syndrome?

Material and methods

The menstrual patterns of women undergoing elective-interval laparoscopic tubal sterilization with Silastic rings (Falope bands) were studied prospectively and longitudinally. A second group of women, whose husbands were undergoing vasectomy, was similarly evaluated as a potential control group. Follow-up rates of

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Table I. Pelvic conditions of the tubal sterilization cohort (n = 389)

Condition	*No. of patients*	*Relative frequency (%)*
Myoma	21	5.4
Edometriosis	6	1.5
Adhesions	27	6.9
Existing pelvic inflammatory disease	16	4.1
Varicosities	5	1.3
Ovarian cyst	8	2.1
Paraovarian cyst	5	1.3
Mixed	10	2.6
Total	98	25.2

81% and 56% respectively resulted in a tubal sterilization cohort of 389 patients and a vasectomy wife cohort of 40 women who were suitable for study. Each patient filled out a questionnaire on the day preceding sterilization and again at 6 months and 1 year after sterilization. The questionnaire called for demographic information and the past medical, surgical, contraceptive, obstetric, and gynecologic history. Patients were asked to provide descriptions of their last three menstrual periods regarding interval between periods, duration of flow, number of days of heavy flow, number of pads or tampons used on the heaviest day of flow, and presence and severity of dysmenorrhea. Patients were also questioned about dyspareunia and noncyclic pelvic pain. A few tubal sterilizations were performed in conjunction with elective dilatation and curettage, but no patients were known to be pregnant within 3 months of the procedure.

Statistical tests of the distributions of six demographic variables in the pretubal sterilization group of 389 women and in the 40 female partners of men scheduled to undergo vasectomy showed the two groups to differ significantly in marital status, educational level, annual income, and race. Because of these differences and the disparity in sample sizes, it was considered statistically appropriate to define the 389 tubal sterilization patients and the 40 female partners of vasectomy patients as independent cohorts and to evaluate the significance of responses in variables, as observed after 6 months (period 1) and 12 months (period 2) following sterilization, in the context of a "before and after" design. Each woman could thereby serve as her own within-cohort control and the independent cohorts could provide a general index of similarity and difference.

The presterilization gynecologic histories of the two cohorts exhibited close resemblance. They differed only in the percent having dilatation and curettage three or more times. The comparative frequency of diabetes, hypertension, pelvic inflammatory disease, malignancy, and medication profiles was similar. The presence of pelvic pathologic conditions, as observed during laparoscopy, could not be documented in women of the vasectomy cohort.

The special difficulties of prospective questionnaire study design posed questions as to the proper choice of analytical techniques. So far as confidence intervals are concerned, we followed a policy of (1) employing χ^2 tests when appropriate, (2) using Ridit transformation on all ordered variables with more than two effective categories, and (3) using the t test whenever applicable. Ridit analysis facilitates intercomparison of factors, avoids the need for arbitrary combinations of categories, and is more powerful than the χ^2 test in detection of shifts in the distributions. In addition, the procedure avoids the usual assumptions of normality.[12] Associations of chosen variables were assessed with Spearman's rho.

At the time of laparoscopic tubal sterilization, several pathologic conditions were observed, and these are presented in Table I. Most of the pelvic adhesions not attributed to chronic pelvic inflammatory disease were due to previous cesarean sections. The mixed category refers to patients with a combination of two or more abnormal findings. There was no difference in menstrual parameters or pelvic pain between the group with pelvic pathologic conditions and the remainder of the study group. Also, there was no difference between those who had concomitant dilatation and curettage and those who did not. Therefore, the entire study group was considered as a whole unit.

Results

Menstrual characteristics. In Table II the cohort status of three quantifiable menstrual parameters are compared to presterilization status after 6 months and 12 months. Six months after sterilization, 145 patients (37.3%) noted an increase in the number of days of menstrual flow whereas 74 (19%) described a decrease, yielding a net increase in 71 patients (18%), with an average increase of 3.8 days per patient. After 1 year a net increase above presterilization status was present in 14.6% of patients, with an average increase of 2.4 days per patient. The increase in duration of menstrual periods for the group as a whole, therefore, does not continue during the second 6 months but rather shows a small tendency to return toward baseline status.

In order to confirm this tendency and gain an individualized, longitudinal perspective, we compared the same menstrual parameters for one time period to the next (category of change, Table II). Period 2 is compared to each patient's status in period 1, which is compared to presterilization status. Since the duration of flow may increase, decrease, or remain unchanged in each time period, nine possibilities emerge. Of the 145 patients who noted an increase in the number of days

Table II. Comparison of patient responses in three menstrual parameters after tubal sterilization (n = 389)

Number of days of usual menstrual period									
Cohort status 6 months after sterilization:	Increase 145 (37.3%), no change 170 (43.7%), decrease 74 (19.0%); average change/patient: +3.8 days/patient*								
Cohort status 12 months after sterilization:	Increase 162 (41.6%), no change 126 (32.4%), decrease 101 (26.0%); average change/patient: +2.4 days/patient*								
Category of change†:	(I1/I2)	(I1/N2)	(I1/D2)	(N1/I1)	(N1/N2)	(N1/D2)	(D1/I2)	(D1/N2)	(D1/D2)
	20	69	56	17	126	27	29	15	30
Number of days of heavy flow									
Cohort status 6 months after sterilization:	Increase 127 (32.6%), no change 176 (45.2%), decrease 86 (22.1%); average change/patient: +3.2 day/patient*								
Cohort status 12 months after sterilization:	Increase 154 (39.7%), no change 118 (30.3%), decrease 117 (30.0%); average change/patient: +1.9 day/patient*								
Category of change†:	(I1/I2)	(I1/N2)	(I1/D2)	(N1/I2)	(N1/N2)	(N1/D2)	(D1/I2)	(D1/N2)	(D1/D2)
	8	62	57	27	118	31	39	46	1
Number of days of usual menstrual cycle									
Cohort status 6 months after sterilization:	Increase 96 (24.7%), no change 249 (64.0%), decrease 44 (11.3%); average change/patient: +0.8 day/patient								
Cohort status 12 months after sterilization:	Increase 147 (37.7%), no change 181 (46.6%), decrease 61 (15.7%); average change/patient: +0.7 day/patient								
Category of change†:	(I1/I2)	(I1/N2)	(I1/D2)	(N1/I2)	(N1/N2)	(N1/D2)	(D1/I2)	(D1/N2)	(D1/D2)
	11	40	45	50	181	18	19	24	1

*Significant increase from presterilization status; $p = 0.05$ (*t* test for paired data).

†I1/I2 = Increase periods 1 and 2, I1/N2 = increase period 1 and no change period 2, I1/D2 = increase period 1 and decrease period 2, etc., over number of patients per category.

of flow during the first 6 months, only 20 described a further increase in the second 6 months, while 56 described a decrease, and 69 maintained the same level of increase. Those patients who noted no change or a decrease in duration of flow initially also experienced no net increase during the second 6 months.

Similar observations were made for the number of days of heavy flow with each menstrual period. The average increase was 3.2 days initially and 1.9 days 1 year after sterilization. Both changes are statistically significant and are corroborated by positive correlation with the number of pads or tampons used on the heaviest day ($r_s = 0.60$).

The interval between menstrual periods tended to lengthen initially and then to level off or decrease slightly. When compared to baseline, the average menstrual cycle was slightly longer after 1 year but not at a statistically or clinically significant level.

The vasectomy cohort was analyzed in a similar manner, and no significant change in any of the three menstrual parameters was observed 1 year after sterilization.

Dysmenorrhea. Table III depicts changes in severity of dysmenorrhea 1 year following tubal sterilization. We chose to omit the 6-month data from the table in order to simplify the display of results. In our questionnaire, mild dysmenorrhea was defined as uncomfortable at some time during menstruation but able to function well, moderate meant unable to function well for 1 day but not confined to bed, and severe meant the inability to function well for more than 1 day or confinement to bed for at least 1 day.

Table III. Changes in severity of dysmenorrhea*

	Dysmenorrhea			
Group	*None*	*Mild*	*Moderate*	*Severe*
Preoperative	221	119	31	18
Postoperative				
None	139	35	4	4
Mild	66	68	9	1
Moderate	10	12	12	5
Severe	6	4	6	8

*Numbers in table represent the number of patients experiencing varying degrees of dysmenorrhea before tubal sterilization and 1 year later.

Of the 221 patients with no preoperative dysmenorrhea, 66 developed mild menstrual cramps. This number was partially offset by the 35 patients who had mild dysmenorrhea preoperatively but none postoperatively. Of the 340 patients who had mild or no dysmenorrhea preoperatively, 32 described moderate or severe dysmenorrhea 1 year after tubal sterilization. They were partially offset by 18 patients whose moderate or severe preoperative dysmenorrhea improved to mild or none. We are thus left with a net number of 14 patients (3.6%) who developed moderate or severe dysmenorrhea 1 year after sterilization. No such changes were noted in the vasectomy group.

Effect of contraception. Because oral contraceptives and intrauterine contraceptive devices may strongly influence menstrual parameters, discontinuing these practices after sterilization may exert an effect inde-

Table IV. Relationship of contraception history to change in menstrual characteristics 1 year after tubal sterilization

Group*	No. of patients	*Days of menstrual flow*					
		Increase		*No change*		*Decrease*	
		Expected	*Observed*	*Expected*	*Observed*	*Expected*	*Observed*
1. OC-P_s, B/N-P_1, B/N-P_2	92	38.3	87	29.8	4	23.0	1
2. IUD-P_s, B/N-P_1, B/N-P_2	43	17.9	20	13.9	12	11.2	11
3. B/N-P_s, B/N-P_1, B/N-P_2	233	97.0	52	61.1	92	60.5	89
4. OC-P_s, B/N-P_1, OC-P_2	15	6.2	2	4.9	13	3.9	0
5. OC-P_s, OC-P_1, OC-P_2	5	2.1	0	1.6	5	1.3	0
6. IUD-P_s, B/N-P_1, OC-P_2	1	0.4	1	0.3	0	0.3	0
Totals	389		162		126		101

*OC = oral contraceptive, IUD = intrauterine device, B/N = barrier or no contraception, P_s = presterilization, P_1 = period 1, P_2 = period 2.

Table V. Noncyclic pelvic pain*

Group	*Pain*			
	None	*Mild*	*Annoying*	*Severe*
Preoperative	258	106	21	4
Postoperative				
None	176	40	2	1
Mild	66	48	4	0
Annoying	13	17	13	1
Severe	3	1	2	2

*Numbers in this table represent the number of patients experiencing varying degrees of noncyclic pelvic pain before and 1 year after tubal sterilization.

pendent of the sterilization process per se. Contraceptive usage is divided into three categories: use of oral contraceptives, intrauterine devices, or neither. The last group is composed of couples using barrier methods, natural family planning, coitus interruptus, or no contraception and is referred to as B/N (barrier or no contraception). In the tubal sterilization cohort, for reasons that are not clear, five of 112 patients continued taking oral contraceptives. Fifteen patients resumed oral contraceptives the second 6 months, presumably for menstrual regulation. All intrauterine contraceptive device users had their device removed shortly before or at the time of sterilization. One intrauterine contraceptive device user took oral contraceptives during the second 6 months. None of the presterilization B/N patients took oral contraceptives after sterilization. The vasectomy cohort follows a somewhat different pattern, since they do not relinquish previous contraception for several months. Prior to their partner's sterilization, 10 members of the vasectomy cohort were taking oral contraceptives, two used intrauterine contraceptive devices, and 28 were classified as being in the barrier or no contraception group.

Table IV documents the relationship of contraceptive history to changes in duration of menstrual flow, number of days of heavy flow, and number of days of the menstrual cycle 1 year after tubal sterilization. Groups 4, 5, and 6 represent small numbers and atypical uses of contraceptive modalities. Groups 1, 2, and 3 are critical to the study.

The first column in each pair of numbers represents the expected frequency of changes in menstrual parameters based on the total study population. The second column gives the actual number of patients experiencing changes. Patients discontinuing the use of oral contraceptives are significantly overrepresented in the subgroup of patients who experience an increase in duration of menstrual flow, increase in number of days of heavy flow, and lengthening of the menstrual cycle. Intrauterine device users were neither overrepresented nor underrepresented. Those patients who never used oral contraceptives or intrauterine contraceptive devices (the barrier or no contraception group) were significantly underrepresented in both increased flow groups, overrepresented in the decreased flow groups, and showed no significant change in the frequency of menstrual periods. Among the 233 patients using barrier or no contraception there are more women who experienced a decrease in duration of flow than an increase (89 compared to 52) and more patients who experienced a decrease in the number of days of heavy flow (98 compared to 55). The majority of patients reported no change in the frequency of menstrual periods, with a similar number experiencing increases and decreases.

Changes in dysmenorrhea paralleled those of menstrual flow and were statistically highly associated. Of the 104 women who experienced an increase of any magnitude, 82 were previous oral contraceptive users, although the expected frequency was only 30. Conversely, in the barrier or no contraception group, more women experienced a decrease in dysmenorrhea than an increase (68 compared to 29).

Days of heavy flow						*Days of menstrual cycle*					
Increase		*No change*		*Decrease*		*Increase*		*No change*		*Decrease*	
Expected	*Observed*	*Expected*	*Observed*	*Expected*	*Observed*	*Expected*	*Observed*	*Expected*	*Observed*	*Expected*	*Observed*
36.4	79	27.9	8	27.6	5	34.8	81	42.8	9	14.4	2
17.0	13	13.0	14	12.9	11	16.2	19	20.0	12	6.7	12
92.2	55	70.7	80	70.1	98	88.0	44	108.4	143	36.5	46
5.9	1	4.5	12	4.5	2	5.7	2	7.0	12	2.3	1
2.0	0	1.5	4	1.5	1	1.9	0	2.3	5	0.8	0
0.4	1	0.3	0	0.4	0	0.3	1	0.5	0	0.2	0
	154		118		117		147		181		61

Table VI. Relationship of contraception to changes in noncyclic pelvic pain after tubal sterilization

	No. of patients	*Status of pain at 12 months*					
		Increase		*No change*		*Decrease*	
1. OC-P_s, B/N-P_1 B/N-P_2	92	24.1	26	56.5	54	11.4	12
2. IUP-P_s, B/N-P_1, B/N-P_2	43	11.3	14	26.4	27	5.3	2
3. B/N-P_s, B/N-P_1, B/N-P_2	233	61.1	57	143.2	146	28.8	30
4. OC-P_s, B/N-P_1, OC-P_2	15	3.9	4	9.2	9	1.8	2
5. OC-P_s, OC-P_1, OC-P_2	5	1.3	1	3.1	3	0.6	1
6. IUP-P_s, B/N-P_1, OC-P_2	1	0.3	0	0.6	0	0.1	1
Total	389	102		239		48	

Noncyclic pain. Patients were asked about pelvic pain occurring at times other than their menstrual period and, if present, to categorize it as mild, annoying, or severe. Most patients who described a change had experienced it in the first 6 months, but there was a slight progression during the second 6-month period. Table V compares severity of noncyclic pelvic pain before sterilization to that described 1 year later. Of the 258 patients who had no pelvic pain before tubal sterilization, 151 (58.5%) remained pain-free for at least 1 year after sterilization. Of the 106 patients who had mild noncyclic pelvic pain before the operation, 18 reported an increase after 1 year and 40 reported a disappearance of pain. The other 48 retained or regained their preoperative pain status. In those patients with annoying or severe preoperative pain there are differing individual patterns but no distinctive change in direction for the group as a whole.

A summary of the observed changes in severity of noncyclic pelvic pain reveals that after 1 year 239 patients (61%) had either regained or retained their presterilization status. One hundred two patients (26%) reported an increase in severity, and 48 patients (12%) reported a decrease. Among the 102 patients reporting an increase, 85% increased one category only and 66 of these changes were from none to mild. Thus, only 17, or 4.4%, of the 389 tubal sterilization patients reported major increases in the severity of noncyclic pelvic pain (i.e., none to annoying or severe in 16 patients; mild to severe in one patient).

Table VI compares the observed and expected frequency of noncyclic pelvic pain in each category of contraceptive usage. There is no significant deviation from expected values, and therefore, unlike dysmenorrhea occurrence, no relationship to previous contraceptive usage. However, tubal sterilization patients of known age (383 patients) showed a positive association between age and noncyclic pelvic pain (Table VII). Increases in noncyclic pain are observed at significantly elevated frequencies in those age categories exceeding 35 years. There were no significant changes in noncyclic pelvic pain in any age category in the small vasectomy cohort.

As mentioned previously, pelvic pathologic conditions ascertained during the sterilization procedure could not be shown to be associated significantly with abnormalities of any menstrual parameter or with noncyclic pain.

Comment

Our study analyzes the results of one method of tubal sterilization, laparoscopic placement of Silastic rings performed in one institution, at a time unrelated to a recent pregnancy. Women whose husbands were

Table VII. Relationship of patient age to change in noncyclic pelvic pain after tubal sterilization

	Status of pain at 12 months						
	Increase		*No change*		*Decrease*		
Age (yr)	*Observed*	*Expected*	*Observed*	*Expected*	*Observed*	*Expected*	*Total*
19-25	1	6.5	17	15.3	7	3.1	25
26-30	11	21.2	51	49.8	19	10.0	81
31-35	30	34.9	86	81.7	14	16.4	133
36-40	41	29.1	70	68.2	3	13.7	111
41-45	12	6.8	10	16.0	4	3.2	26
46-50	5	1.8	2	4.3	0	1.0	7
No data	2	1.6	3	3.7	1	1.0	6
Total	102		239		48		389

undergoing vasectomies were recruited as a control group. Because of the small number of these wives; their different demographic characteristics, and the lag time in giving up contraceptive modalities, the vasectomy cohort could not be used as a suitable control group. Yet comparisons of trends and tendencies could be made to help corroborate our data. We feel that for several reasons our investigational design is valid for answering the principal question. Our study is prospective and follows each patient during a continuum of 1 year, enabling each patient to serve as her own before and after control. We document the presence or absence of pelvic pathologic conditions, account for demographic characteristics and past medical, surgical, and gynecologic problems, and carefully consider the role of the giving up of previous contraceptive practices.

We assessed menstrual flow according to duration, number of days of heavy flow, and the use of pads or tampons. Comparing the number of pads or tampons used by one woman to the number used by other women cannot accurately distinguish differences in flow. However, since women tend to establish their own pattern of menstrual protection, changes in the use of pads or tampons by the same woman is a good indicator of changes in flow. Similarly, description of changes in pelvic pain would seem to be valid if the intensity of the pain was documented prospectively.

The vagaries of patient responses to questionnaires also play a role in analyzing data of this type. These variations should, however, balance themselves so that the number of women reporting increases in any parameter should be similar to the number reporting decreases if the sterilization procedure itself did not have a primary effect. We therefore describe a net increase in flow or pain only when the number of women reporting an increase exceeds the number reporting a decrease. Our data tend to support this hypothesis, since parameters that would not be expected to change show similar numbers of increases and decreases.

Analysis of the data for the entire tubal sterilization cohort shows a statistically significant increase in menstrual flow and dysmenorrhea, which occurs within the first 6 months following tubal sterilization, then tends to return toward, but never reaches, baseline presterilization values. When prior use and discontinuation of oral contraceptives are factored out, no increase but rather a nonsignificant decrease in menstrual flow and dysmenorrhea is observed.

It is apparent therefore that tubal sterilization did not significantly affect menstrual function, but that discontinuing the use of oral contraceptives is the key factor resulting in an increase in menstrual flow and dysmenorrhea. It is well recognized that many patients taking oral contraceptives have less menstrual flow and less dysmenorrhea than before taking them. It is also quite likely that many oral contraceptive users were physician-selected or self-selected initially, not only for contraception but to alleviate heavy menstrual flow, dysmenorrhea, or dysfunctional uterine bleeding. The increases reported are probably a return to what the baseline menstrual parameters would have been if these women had not taken the pill.

Only two hysterectomies (0.5%) occurred in our entire group, both in previous users of oral contraceptives. One of them was admitted as an emergency patient for sudden, profuse uterine bleeding following 6 months of amenorrhea. The other patient had intractable dysfunctional uterine bleeding that began immediately following tubal sterilization. No pathologic conditions were found in either uterus.

Surprisingly, intrauterine contraceptive device users did not report a significant decrease in menstrual flow or dysmenorrhea. Perhaps this group of 41 patients is composed of women who were successful long-time users of intrauterine contraceptive devices with no ill effects who chose sterilization electively rather than because of problems related to the device. Those who had problems may well have changed to a different form of temporary contraception rather than sterilization.

Significant dyspareunia was present in less than 3%

of patients preoperatively and did not change appreciably during the study. The interval between menstrual periods increased during the first 6 months but returned close to baseline levels after 1 year, probably representing a self-limited delay in resumption of ovulation following discontinuation of oral contraceptives.

Noncyclic pelvic pain behaves differently than dysmenorrhea. Changes which occur are not related to previous contraceptive usage but are positively correlated with age. Patients with prior pelvic pain did not show any significant net increase regardless of degree of severity. In the group with no recent history of pelvic pain before sterilization there is a significant number who reported the presence of pain at 6 months and 1 year, but the majority characterized it as mild. Major increases in noncyclic pain described as annoying or severe occurred in only 4.4% of the study cohort and were strongly associated with age. Of the 17 patients reporting a major increase in pain, 15 were >38 years of age.

The development of pain in a small percentage of people who had pelvic surgery may be expected, but the strong association with increasing age cannot be readily explained. Pain does not correlate with parity or with an operative description of pelvic varicosities and therefore remains an enigma.

At the conclusion of the study all 389 patients were asked if they had regrets about the operation. Forty patients (10%) indicated regret, eight because of menstrual problems and seven because of pelvic pain. Ten women expressed a desire to have another child, and three wished they had retained the option of having a child even though they did not want to have one at the present time. Twelve patients had reasons which can be classified as psychological.

In summary, changes in menstrual flow, menstrual cycles, dysmenorrhea, dyspareunia, and noncyclic pelvic pain were studied following interval laparoscopic tubal sterilization by means of Silastic rings. Conclusions about other means of sterilization techniques cannot be drawn from this study, but most of the reports in the literature showing significant increases in menstrual parameters were carried out on patients who underwent tubal fulguration. In our study, menstrual flow and dysmenorrhea were strikingly increased but only in patients who discontinued oral contraceptives. When the role of previous contraception was carefully discounted, no change in menstrual parameters occurred. Thus the so-called post–tubal sterilization syndrome, at least following tubal banding, would seem to be a misnomer. The symptoms that have been associated with that syndrome occur as a result of discontinuing oral contraceptives rather than as a result of the tubal sterilization per se.

A small percentage of women over age 38 develop significant noncyclic pelvic pain. Why age predisposes women to pelvic pain cannot be explained at present.

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Adhesion formation and histologic reaction with polydioxanone and polyglactin suture

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A suture material associated with a minimal inflammatory response might be expected to induce less frequent and less severe peritoneal adhesions. A comparison between polydioxanone and polyglactin 910 suture was performed in a rabbit model. Ten sexually mature virgin female New Zealand White rabbits underwent laparotomy and bilateral incisions into the distal uterine cavities. The serosa of the left uterine horn was always reapproximated with polyglactin 910 suture whereas the right uterine horn was repaired with polydioxanone suture. All animals were put to death 28 days later. An adhesion score was given for each uterine horn. Representative sections were obtained for histologic review. Similar histologic responses were found in both groups. No significant difference was noted in adhesion scores between the two sutures. The present study cannot justify the use of one of these sutures over the other with regard to adhesion formation or tissue reaction. (AM J OBSTET GYNECOL 1985;151:20-3.)

Key words: Adhesions, sutures

The relationship of chronic tissue reactivity and peritoneal adhesions to the type of suture material used is controversial. A suture material associated with a minimal inflammatory response might be expected to induce less frequent and less severe peritoneal adhesions. DeCherney and Laufer[1] reported less adhesion formation and chronic inflammatory reaction with polydioxanone suture than with polyglactin (Vicryl) suture in a rat model. In a rabbit model, Delbeke et al.[2] found no difference in histologic reaction between the above two suture materials but did not report adhesion induction in the animals.

Encouraged by previous reports of reduced inflammatory response[1, 3, 4] and adhesion formation,[1] we performed a comparison between polydioxanone and polyglactin 910 suture in a previously studied rabbit model.[5]

Material and methods

Ten sexually mature virgin female New Zealand White rabbits were individually caged and received identical care. The rabbits weighed between 3 and 4 kg at the time of operation. Each animal underwent aseptic laparotomy through a lower midline abdominal incision. Ketamine hydrochloride (Vetalar, Parke-Davis) and xylazine hydrochloride (Rompun, Haver-Lockhart) were given intramuscularly to induce and maintain anesthesia. No prophylactic antibiotics were used. The uterine horns were externalized and infiltrated bilaterally with a dilute solution of vasopressin (4 pressor units per 20 ml of normal saline) to reduce bleeding. Bilateral incisions 4 cm long were made into the distal uterine horns with a scalpel. The incisions were then meticulously reapproximated with running serosal stitches of either polydioxanone or Vicryl suture. All suture used on the uterine horns was 6-0 caliber. The needle was a precision point–reverse cutting type (P-1, Ethicon) in both groups. The left uterine horn of each rabbit was always reapproximated with Vicryl suture whereas the right horn was repaired with polydioxanone suture. No magnification was used in either group and tissue handling was uniform. No intraperitoneal irrigation was performed. The abdominal incisions were closed with two layers of 3-0 chromic suture.

All animals were put to death 28 days after operation. An adhesion score (Table I) was given for each uterine horn by M. R. N. and G. L. H., who agreed in each case on an adhesion score for each horn.

Matching representative sections were obtained from six of the 10 animals for histologic review. The extent of adhesions in the other four animals prevented successful en bloc removal of the uterine horns to evaluate the site of repair. The tissue sections were then placed

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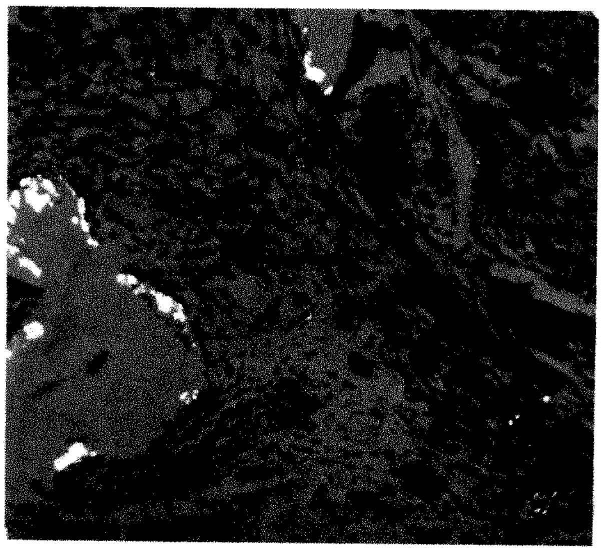

Fig. 1. Histologic section revealing residual polydioxanone suture at 28 days with polarization analysis.

Table I. Adhesion scoring system

Score	*Criterion*
0	No macroscopic adhesions observed
1	One filmy macroscopic adhesion
2	Multiple macroscopic filmy adhesions
3	One dense macroscopic adhesion with or without additional macroscopic filmy adhesions
4	Multiple dense macroscopic adhesions with or without additional filmy adhesions

in formalin solution and forwarded to W. L. B. for histologic evaluation. The pathologist was not aware of the name or characteristics of each suture at the time of evaluation but was informed that two suture materials were being evaluated. Five to seven tissue sections from each uterine horn were microscopically evaluated. The tissue sections were stained with hematoxylin and eosin as well as Masson's trichrome stain. Multiple parameters were assessed as described in Table II. These included degree of chronic inflammation, giant cell response, degree of serosal adhesions, tissue edema, amount of glandular entrapment within the muscularis layer, and telangiectasia. The degree of persistent suture material was also evaluated. Identification of residual suture material was aided by polarization analysis as seen in Fig. 1. The parameters were graded on a scale of 1+ to 3+ with the assigned grade dependent upon the degree of deviation from normally expected tubal histologic features.

A χ^2 analysis was performed on the adhesion scores presented in Table III. An additional χ^2 analysis was done to discriminate between filmy (scores of 1 or 2) and dense (scores of 3 or 4) adhesions noted in the two suture groups.

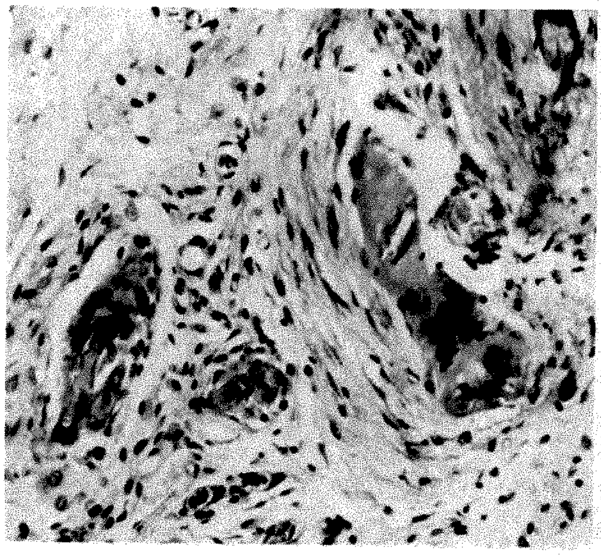

Fig. 2. Histologic section with hemotoxylin and eosin stain at 28 days with polydioxanone suture.

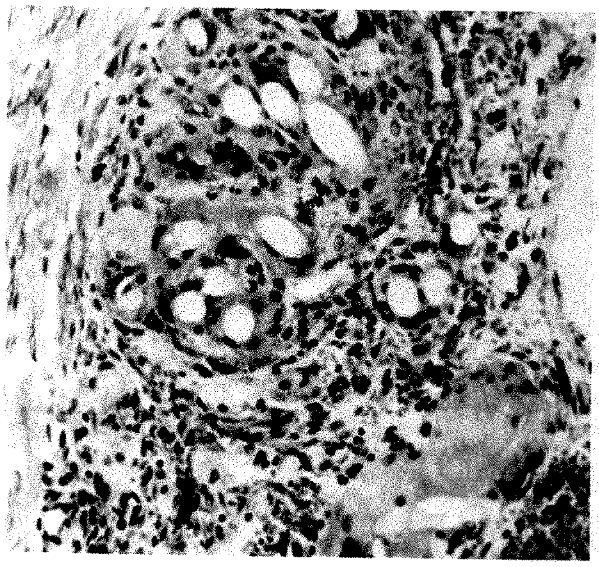

Fig. 3. Histologic section with hemotoxylin and eosin stains at 28 days with Vicryl suture.

Results

No significant difference was noted in adhesion scores between the two sutures (Table III). There was also no significant difference in analysis for the presence or absence of dense adhesions, that is, adhesion scores of 3 or 4.

Similar histologic responses were found in both groups (Figs. 2 and 3). No significant differences were noted in any of the stated parameters (Table II). Remnants of suture could be detected in at least one of the

Table II. Parameters of tissue reaction to polydioxanone and Vicryl suture at 28 days

Animal No.	*Chronic inflammation*	*Giant cells*	*Serosal adhesions*	*Edema*	*Telangiectasia*	*Entrapped glands*
V_1	0	0	0	0	0	0
V_2	+1	+1	0	0	+2	0
V_3	0	+2	0	+2	0	0
V_4	+1	+2	+2	+2	+2	+1
V_5	0	+2	+1	+1	0	+1
V_6	+1	+2	+2	+1	0	0
P_1	0	0	0	+2	+2	0
P_2	0	0	+1	+2	+2	0
P_3	0	0	0	+2	+1	0
P_4	0	0	0	+1	+1	0
P_5	+2	+2	+2	+2	0	+1
P_6	+2	+2	+1	+2	+1	0

The parameters were graded on a scale of 0 to 3+.

Table III. Relationship of suture material to adhesion score

Suture	*Adhesion score*				
	0	*1*	*2*	*3*	*4*
Polydioxanone	1	0	1	6	2
Vicryl	2	1	1	1	5

five to seven sections of uterine horn in each animal. In most cases where residual material was visualized with polarization analysis, the giant cells had effectively phagocytized the suture. One animal (P_6) had large amounts of extracellular polydioxanone but not Vicryl suture that was microscopically polarizable.

It appears that the most common responses involve intraplical and periplical edema and telangiectasia of varying degree. In more advanced cases, adhesions form on the serosal surface and are often accompanied by a giant cell response. This chronic inflammatory response also is accompanied by lymphocytes and single histiocytes as well as a few plasma cells. Serosal edema and chronic inflammatory response precede early fibrosis. With increasing degrees of serosal fibrosis, the chronic inflammatory infiltrate subsides and the giant cell accumulation is left. In summary, no significant microscopic differences were noted in rabbit uterine horn responses to polydioxanone and Vicryl suture at 28 days after operation.

Comment

This study cannot confirm that a significant difference exists in the histologic reactivity of these two suture materials and thus verifies the findings of Delbeke et al.[2] Similarly, no difference in adhesion induction was noted.

It would be expected from previous work that adhesion formation is present well within the time frame of this study.[6] It is doubtful that a longer observation period would demonstrate an increased incidence of adhesions in either group.

Previous studies have confirmed that adhesion formation is reduced when peritoneal injury and ischemia are minimal.[6] The peritoneal and vascular injury produced by any suture is a major cause of adhesion induction. Therefore, as demonstrated previously, suture of low caliber, as is commonly used in conservative or reconstructive surgery, should be less frequently associated with adhesion induction by reducing the development of tension and, therefore, ischemia along the lines of incision.[5] Likewise, atraumatic needles would also be expected to reduce peritoneal injury. This injury induced by large or otherwise traumatic needles is known as the "trocar effect." Both of the above variables, caliber of suture and needle type, were also investigated in another study that did not include polydioxanone.[7] That study addressed the histologic reaction to various microsutures. An analysis of adhesion formation was not discussed. The conclusion reached was that there was a direct relationship between suture caliber and tissue reaction. The degree of tissue reaction did not vary with type or size of needle used. The sizes of the needles used were all <145 μm. The results of the present study cannot justify the use of one of these sutures over the other with regard to adhesion formation or tissue reaction although other factors may lead one to choose one suture over the other. One such factor that we appreciated is the better handling characteristics of the polydioxanone suture used in this study.

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Carbon dioxide laser for combination excisional-vaporization conization

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Extensive cervical intraepithelial neoplasia may simultaneously involve large areas of the ectocervix, even extending to the vaginal fornices, as well as the endocervical canal. In such instances conventional sharp knife conization would result in virtual removal of the cervix if the surgeon wished to completely circumscribe the entire lesion. By combining a narrow carbon dioxide laser excisional conization with ectocervical vaporization, cervical intraepithelial neoplasia may be eradicated by a virtually bloodless surgical procedure and fertility may be preserved. Sixty-one combination conizations with 6 months to 4 years of follow-up are reported. In 95% of the cases biopsy demonstrated cervical intraepithelial neoplasia in three or more ectocervical quadrants, and 16% had extension of disease into the vagina. The technique of combination conization uses 20 to 30 W of power and $^1/_2$ to 1 mm spots for excision and 10 to 15 W and 1 to 1.5 mm spots for vaporization. The average time to complete the entire procedure was 21 minutes. In three of 61 cases endocervical margins were positive; there were no instances of persistence of neoplasia at either the ectocervical or the vaginal margins. Four complications were observed, all related to delayed bleeding; however, only two patients required the placement of a suture. The volume of tissue removed by a large excisional conization is more than two times greater than that of a laser combination conization. (Am J Obstet Gynecol 1985;151:23-7.)

Key words: Laser, laser excision, conization, combination cone, carbon dioxide laser combination cone

Cervical conization is a recognized standard for both the diagnosis and the treatment of premalignant disorders of the cervix.[1-4] Insofar as its application for diagnosis is concerned, the specimen provided to the pathologist must be large enough to afford satisfactory orientation in order to avoid tangential cutting while providing multiple study samples ranging from 16 to 150 sections. The major conclusion to be obtained from a cone specimen is whether invasion is present. Additional supplementary data relate to neoplastic involvement of the ectocervical or endocervical margins. Reduced to the lowest common denominator, the cone biopsy, the directed punch biopsy, and the endocervical curettage are simply methods of sampling. The greater the number of sample sections available, the less the chance of diagnostic error and vice versa. Clearly, the cone specimen provides the most plentiful mass of tissue for sampling; therefore, diagnostic errors are diminished.

The most accurate cervical conizations are obtained when the operation is performed in conjunction with colposcopy. The magnified vision provided by the microscope and the accentuation with acetic acid allow accurate determination of the ectocervical extent of the conization. Unfortunately, there is no accurate method to absolutely guarantee clearance of the endocervical margins. Recently, endoscopic examination of the canal has been advocated to visually determine the extent of progression of neoplasia into the endocervical canal.[5]

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Table I. Geographic extent of cervical intraepithelial neoplasia in patients treated by carbon dioxide laser combination conization

Extent	*n*	%
One quadrant	0	0
Two quadrants	3	5
Three quadrants	11	18
Four quadrants	37	61
Vaginal forniceal extension	10	16

Several investigators have established conization as an efficacious method for the cure of cervical intraepithelial neoplasia.[6-8] The cervical conization operation is conventionally performed with a sharp knife, usually with the patient under general anesthesia. The procedure is associated with bleeding and requires the placement of sutures to quench the flow of blood. The Sturmdorf suture was used in the past,[9] and this older methodology has even persisted to the present time, although it frequently results in postoperative cervical stenosis. Interrupted figure-of-eight sutures represent a more preferred contemporary technique of repair. Sharp knife conization should be performed in a hospital setting because the technique may be associated with immediate complications such as bleeding or perforation, as well as delayed complications such as bleeding, infection, stenosis, incompetency, and reduced fertility.[4, 10]

The advent of the carbon dioxide laser has allowed this instrument to be directly substituted for the scalpel for the performance of excisional conization[11, 12] as well as for cervical vaporization procedures.[13-15] Although the two laser techniques are similar from the standpoint that a measurable volume of cervical tissue is removed, the former operation provides a specimen for pathologic examination whereas the latter provides nothing since the tissue literally disappears. The indications for excisional laser versus vaporization procedures have been enumerated elsewhere.[13] The advantages of the laser versus conventional techniques are: (1) The laser conization is performed entirely under microscopic guidance with the use of a micromanipulator; (2) the laser conization reduces blood loss since the light beam seals small vascular channels, thereby eliminating in most cases the need for sutures; (3) an "endocervical button" may be shaped to diminish the chances for subsequent stenosis; (4) healing proceeds rapidly; (5) virtually every operation is performed on an outpatient basis.

For the above reasons, once a gynecologic surgeon becomes adept at the laser technique, he/she will usually prefer it in place of the scalpel for the future performance of this operation. Additionally, since the laser excisional conization involves the use of only fine plastic surgery–type skin hooks for the manipulation of the specimen, there is minimal trauma of the cervical mucosa to be examined by the pathologist. Not infrequently, the cervix is affected with extensive neoplasia that involves 50% of the surface area of the portio or may spread geographically to involve the entire ectocervix and the vaginal fornices. In such cases, the surgeon is faced with an enigma, since excision of the entire lesion by conization would result in the de facto removal of not only the diseased epithelium but most of the cervical stroma and infertility would result. On the other hand, performance of a less radical operative excision would leave residual disease behind with inevitable persistence and possible progression. A new and specific carbon dioxide laser technique provides a simple option by the exigency of combining the laser excisional conization and the laser vaporization techniques. This procedure is best termed for descriptive purposes the combination conization.

Material and methods

Sixty-one combination conizations of a total of 215 laser excisional conizations were performed at Mt. Sinai Hospital, Hartford, Connecticut, and Greater Baltimore Medical Center, Baltimore, Maryland, between the years 1979 and 1983. Only those cases with follow-up cytologic and colposcopic data available for 6 months to 4 years were included in this series. The patients' ages ranged from 16 to 55 years with a mean age of 33 years. Fifty-three women were white; the remainder were black. The severity of cervical intraepithelial neoplasia was skewed toward more severe grades of intraepithelial neoplasia, that is, 96.7% having preoperative biopsy proved grade 2 (28) or 3 (31) cervical intraepithelial neoplasia. Fifty-eight cases (95%) had proved cervical intraepithelial neoplasia in three or more quadrants; 10 cases (16%) had contiguous extension of neoplasia into the vagina. Fifty-nine patients had disease that extended into the endocervical canal and additionally showed extensive portio involvement. Ten women had Papanicolaou smear–biopsy disparity as one indication for the excisional conization (Table I). Every conization was performed in the hospital on an outpatient basis with the patient under either general inhalation (55 cases) or paracervical block (six cases) anesthesia. The criteria for selecting combination conization rather than simple excisional conization were: (1) neoplasia involved >50% of the surface of the ectocervix; (2) there was simultaneous extension of neoplasia into the endocervical canal or Papanicolaou smear–biopsy disparity; (3) the quantity of stroma to be removed was judged so great that the risk of future infertility was substantial.

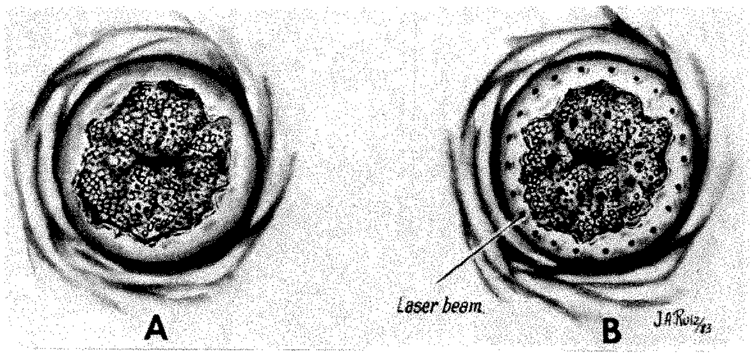

Fig. 1. *A*, An extensive abnormal transformation zone is illustrated. Cervical intraepithelial neoplasia extends into the endocervical canal and far out onto the portio. Conventional conization would effectively result in removal of the cervix and infertility. *B*, With a low-power–density laser beam a narrow conization pattern is outlined. A second circle of dots is outlined extending 5 mm beyond the peripheral boundaries of the ectocervical extension.

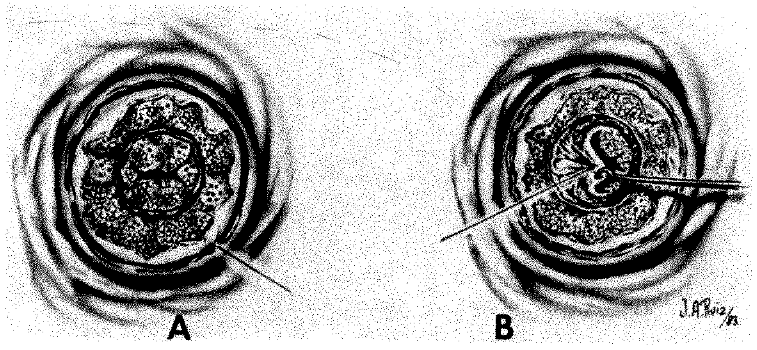

Fig. 2. *A*, Power density is raised to 8000 W/cm^2 as the beam cuts deeply into the underlying stroma. *B*, Fine long-handled laser skin hooks place traction on the conization specimen enabling the laser beam to sharply cut to a depth of 1.5 to 2 cm.

Preoperative evaluation. Each woman had one or more cytologic and colposcopic examinations together with multiple sampling of the ectocervical margin by means of Wittner or Kevorkian biopsy clamps. These biopsies were performed in order to ensure that no invasive disease was present at the ectocervical limits of the lesion. The patient was then scheduled to enter the hospital for a laser combination conization.

Technique. Lateral cervical figure-of-eight traction sutures were placed at the 3 and 9 o'clock positions. The extent of the narrow conization was outlined at a low-power density (500 W/cm^2) with intermittent spots measuring 1.0 mm in diameter (Fig. 1, *A* and *B*) which were then connected. The extent of the vaporization to be performed was similarly marked out peripherally on the cervix or in the vagina. A 1 : 30 solution of dilute vasopressin was injected into the substance of the cervix according to the trace marks with a 25-gauge needle attached to a tuberculin syringe. The vasopressin solution (5 to 7 ml) was injected. The latter solution produced vasospasm in vessels >1 mm in diameter resulting in laser sealing rather than sharp sectioning of the channel. (The laser will usually seal vessels up to $\frac{1}{2}$ mm in diameter.) Power was turned up to 20 to 30 W, the laser spot was reduced to 0.5 to 1 mm in diameter (power density = 8000 to 12,000 W/cm^2), and the cone incision was retraced to obtain deeper depth into the underlying stroma (Fig. 2, *A*). Fine plastic surgery skin hooks were placed into the wound to produce traction and opposing countertraction as the laser beam cut even deeper into the cervical stroma (Fig. 2, *B*). When a depth of 1.5 to 2 cm was obtained, the traction hooks were moved circumferentially around the cervix until a cylinder of cervix was freed (Fig. 3, *A*).

Next the cylinder 1 cm in diameter was pulled to one side as the scalpel cut the cervix free at its endocervical margin (Fig. 3, *B*). With a brush-vaporization technique, an endocervical button was excavated. This margin was carefully examined colposcopically for possible neoplastic involvement. Finally, the power was reduced to 10 to 15 W and the spot was enlarged to 1.0 to 1.5 mm (700 to 1500 W/cm^2). Vaporization of the remaining ectocervix was performed to a depth of 5 to 7 mm with a 3 mm peripheral margin (Fig. 4, *A* and *B*). If

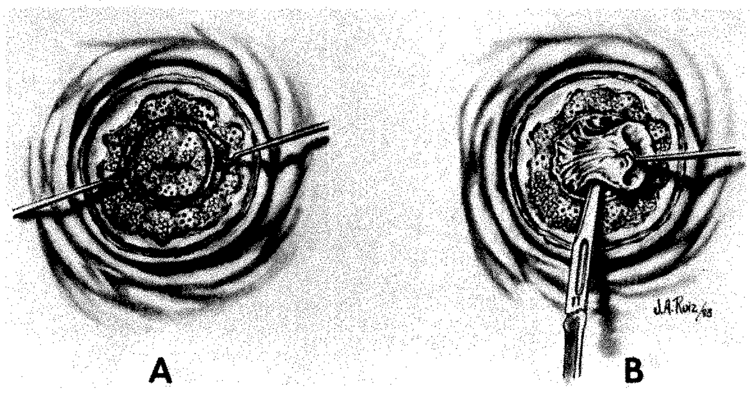

Fig. 3. *A*, A cylinder of cervix has been cut free from the neighboring cervical tissue. *B*, A sharp scalpel blade frees the cylinder of cervix at the endocervical margin, eliminating the risk of tissue distortion secondary to heat.

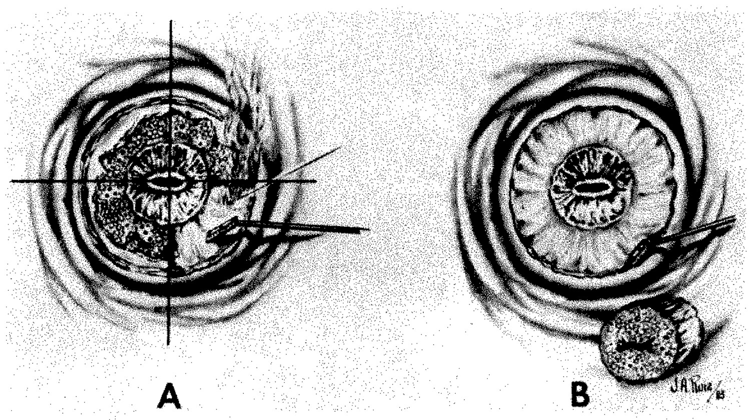

Fig. 4. *A*, Power density is reduced and vaporization is performed to a depth of 5 to 7 mm, thereby eliminating ectocervical disease. Vaporization may be continued into the vagina if necessary. *B*, The combination conization has been completed. The central core of cervical tissue is sent to the pathology laboratory for evaluation. The extensive area of cervical intraepithelial neoplasia has been eliminated; bleeding is minimal, and no sutures are required.

the lesion extended into the fornices, the vaginal extension was vaporized to a depth of 2 mm with a 3 to 5 mm peripheral margin. The end result was a cervix with a deep central core of tissue removed and the remainder vaporized to a depth of 5 to 7 mm. The excisional cone specimen was sent for pathologic examination.

Results

In three of 61 cases, endocervical margins were positive on the excision specimen and were considered failures of cure. No patient had persistence or recurrence at the ectocervical or vaginal margin. When the endocervical margin was involved, a recommended procedure was to obtain additional tissue within the office setting by means of the laser and a long-handled fine hook. This technique attempted to delineate a clear margin. Follow-up with cytologic smears and biopsy was carried out every 6 months, and reconization was performed if long-term persistence was demonstrated.

Three fifths of the patients demonstrated cervical intraepithelial neoplasia involving all four quadrants of the conization specimen and 10 (16.4%) had involvement of one or more of the vaginal fornices (Table I). None of the latter had multicentric involvement of the vagina. If multifocal disease existed in the vagina, these cases were considered primary vaginal intraepithelial neoplasia and were placed in a separate category, that is, not considered extension of cervical intraepithelial neoplasia into the vault.

The average time to complete the procedure after vasopressin injection approximated 21 minutes with the excisional procedure requiring an average time of 10 minutes and the vaporization 11 minutes.

Complications. No patient developed immediate hemmorrhage. Four women had sufficient postoperative bleeding to require examination which was done between 4 and 9 days after operation. Two patients had a generalized ooze from the wound bed that was treated with an application of Monsel's solution. One patient (1.6%) had sustained arterial bleeding from a single vessel which required the placement of a figure-of-eight Vicryl 3-0 suture. Another woman had bleeding at the site of one lateral traction suture, which had torn through the mucosa, thus necessitating resuturing. No infections were identified, no stenosis was observed, and no cases of invasive carcinoma were identified within the minimum 6-month period of follow-up.

Comment

Widespread gross disease reflects extensive microscopic involvement and likewise mirrors more severe grades of neoplasia, that is, in this series 96.7% of the cases were grade 2 or 3 cervical intraepithelial neoplasia. Prior to the advent of the carbon dioxide laser, complex excisional procedures were required for the eradication of geographically extensive cervical intraepithelial neoplasia and often not only proved bloody but led to functional deformity.

The combination conization offers a reasonable solution to a difficult problem, that is, the excisional portion provides the pathologist with an adequate sample to study and the vaporization portion effectively eliminates disease in the ectocervix and vagina.

Although extensive amounts of tissue are removed, substantial cervical stroma is saved by the combination technique. The volume of tissue removed by a 1.5 cm (length) by 2.0 cm (width) excisional cylinder would total 4.71 cm^3 as compared to 2.26 cm^3 when a narrow 1.5 cm (length) by 1.0 cm (width) excision is coupled with a 0.5 cm (length) by 1.0 cm (width) vaporization. Complications have been exceedingly few in this series. The most difficult problem insofar as management is concerned relates to that of residual endocervical disease after the excisional conization. This may be dealt with at the 48-hour postconization visit by means of further tissue excision or by endocervical endoscopy (to define the conization limits) at the time of the original procedure.

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Low-dose oral contraception and blood pressure in women with a past history of elevated blood pressure

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Sixty-one women with a past history of elevated blood pressure (high-risk group) associated with oral contraceptive use (27 patients), unknown etiology (17 patients), or preeclampsia (17 patients) used a low-dose oral contraceptive containing 0.4 mg of norethindrone and 35 μg of ethinyl estradiol for 3 to 24 months. The highest blood pressures recorded in the past were 141.0 ± 14.9 (mean ± SD) mm Hg systolic and 98.3 ± 8.0 diastolic. Sixty-one women without a past history of elevated blood pressure (low-risk group), selected from a pool of 616 low-risk patients, were matched with the high-risk group for race, initial age, initial body weight, duration of use, history of smoking, and family history of hypertension. The blood pressures in the high-risk group before and after oral contraceptive therapy were significantly higher than those in the low-risk group. Despite these differences, the mean systolic and diastolic blood pressures in these two groups of women using the low-dose oral contraceptive did not rise when compared with their own baseline blood pressures. Five high-risk patients (8.2%) discontinued therapy because of the redevelopment of hypertension; however, blood pressures at discontinuance were comparable to the previous highest blood pressures. (Am J Obstet Gynecol 1985;151:28-32.)

Key words: Oral contraceptive, blood pressure, hypertension, low-dose oral contraception

One of the significant side effects of oral contraceptives containing estrogens and progestogens is the development of hypertension (>140 mm Hg systolic and >90 mm Hg diastolic).[1, 2] Fisch and Frank,[3] analyzing the data from 13,358 women, reported the incidence of hypertension for current oral contraceptive users to be 3.8%, while for never users and past users, the rate was between 1% and 1.5%.[3] Additionally, oral contraceptive users have had higher mean blood pressures than their baseline levels or those of nonusers, that is, 3 to 7 mm Hg systolic pressure and 1 to 3 mm Hg diastolic pressure.[3-6]

Factors such as increased age, body weight,[7] and a history of personal[3] or parental hypertension[6] have been associated with elevated blood pressure during oral contraceptive therapy. Race is also a significant variable in the prevalence of hypertension.[8] Although Fisch et al.[9] reported no association between blood pressure and oral contraceptive formulation or dose, their study concerned the use of oral contraceptives containing more than 0.5 mg of a 19-nortestosterone derivative and more than 50 μg of an estrogen.[9]

A prospective study with the use of an oral contraceptive of 0.4 mg of norethindrone and 35 μg of ethinyl estradiol reported no cases of hypertension among 700 women with up to 44 months of use but found a statistically significant increase in mean systolic blood pressure of <3.0 mm Hg.[10] With the same low-dose oral contraceptive, we have shown that the increase in mean systolic blood pressure in the low-dose group was lower than that observed in the groups using higher-dose oral contraceptives containing 1 mg of a progestogen and 50 μg or more of an estrogen.[11] These data suggest that the dosage of sex steroids in oral contraceptive preparations is an important determinant in the elevation of mean blood pressure and occurrence of hypertension among oral contraceptive users. While those two studies[10, 11] of low-dose oral contraceptives enrolled primarily normal women, the present report concerns the effect of the same low-dose preparation on blood pressure in a group of patients with a past history of elevated blood pressure.

Subjects and methods

A prospective study of 677 women using an oral contraceptive containing 0.4 mg of norethindrone and 35

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Table I. Systolic blood pressures in two groups of women using low-dose oral contraceptive*

	Blood pressure (mm Hg) (mean ± SD)		
	Low risk	*High risk*	*Probability*
Baseline	113.8 ± 10.2 (61†)	124.3 ± 10.9 (60)	$p^r < 0.0001$
3 mo	111.8 ± 11.7 (61)	123.3 ± 10.1 (60)	p^t = NS
Baseline	112.0 ± 12.6 (12)	126.9 ± 11.6 (21)	$p^r < 0.0005$
6 mo	113.0 ± 7.1 (12)	124.1 ± 9.2 (21)	p^t = NS
Baseline	115.4 ± 10.0 (42)	123.6 ± 10.1 (34)	$p^r < 0.05$
9 mo	114.8 ± 13.8 (42)	117.2 ± 11.8 (34)	$p^t < 0.05$
Baseline	112.2 ± 9.5 (36)	124.6 ± 10.4 (34)	$p^r < 0.0001$
12 mo	113.3 ± 11.3 (36)	121.9 ± 8.2 (34)	p^t = NS
Baseline	114.6 ± 9.9 (14)	125.6 ± 10.5 (27)	$p^r < 0.0005$
18 mo	110.1 ± 10.6 (14)	120.3 ± 9.0 (27)	$p^t < 0.05$
Baseline	114.0 ± 11.0 (10)	124.7 ± 8.9 (15)	$p^r < 0.01$
24 mo	112.6 ± 8.2 (10)	119.3 ± 12.2 (15)	p^t = NS

p^r = Probability with risk as a factor in two-way analysis of variance of paired observations.
p^t = Probability with time as a factor in two-way analysis of variance of paired observations.
*Oral contraceptive containing 0.4 mg of norethindrone and 35 μg of ethinyl estradiol.
†Number of patients.

Table II. Diastolic blood pressures in two groups of women using a low-dose oral contraceptive*

	Blood pressure (mm Hg) (mean ± SD)		
	Low risk	*High risk*	*Probability*
Baseline	75.2 ± 8.4 (61†)	85.7 ± 9.5 (60)	$p^r < 0.0001$
3 mo	72.0 ± 7.4 (61)	84.0 ± 8.9 (60)	$p^t < 0.0005$
Baseline	76.2 ± 9.4 (12)	89.5 ± 8.3 (21)	$p^r < 0.0001$
6 mo	72.8 ± 7.7 (12)	84.3 ± 8.7 (21)	$p^t < 0.05$
Baseline	75.1 ± 8.3 (42)	84.7 ± 9.5 (34)	$p^r < 0.0001$
9 mo	74.8 ± 7.9 (42)	80.0 ± 8.7 (34)	$p^t < 0.05$
Baseline	74.1 ± 7.4 (36)	84.9 ± 10.3 (34)	$p^r < 0.0001$
12 mo	72.0 ± 9.2 (36)	82.0 ± 6.8 (34)	p^t = NS
Baseline	76.9 ± 7.3 (14)	86.1 ± 10.6 (27)	$p^r < 0.0005$
18 mo	70.7 ± 6.3 (14)	80.0 ± 8.6 (27)	$p^t < 0.001$
Baseline	78.6 ± 3.9 (10)	84.8 ± 10.0 (15)	$p^r < 0.05$
24 mo	72.2 ± 5.8 (10)	79.7 ± 10.9 (15)	$p^t < 0.005$

p^r = Probability with risk as a factor in two-way analysis of variance of paired observations. p^r = Probability with time as a factor in two-way analysis of variance of paired observations.
*Oral contraceptive containing 0.4 mg of norethindrone and 35 μg of ethinyl estradiol.
†Number of patients.

μg of ethinyl estradiol was undertaken from June, 1979, through June, 1983. These women were selected primarily because of their request for oral contraceptives and their willingness to return for follow-up of blood pressure determinations. Sixty-one women with a past history of elevated blood pressure (high-risk patients), seen consecutively and requesting oral contraception, were placed on a regimen of the same low-dose oral contraceptive. A history of elevated blood pressure was defined as a blood pressure recorded in the past ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic. Of the 61 patients, six patients with a diagnosis of preeclampsia had persistent elevation of blood pressure more than 6 weeks post partum, 11 patients had a history of preeclampsia, and 27 patients developed elevated blood pressure while using other types of oral contraceptives (24 patients using oral contraceptives containing 1 mg of progestogen and ≥50 μg of estrogen and three patients using oral contraceptives containing 0.5 mg of progestogen and 35 μg of estrogen). Elevated blood pressure in the remaining 17 patients was found to be unrelated to pregnancy or oral contraceptive use.

Sixty-one women with no history of elevated blood pressure (low-risk patients) were matched with the 61 high-risk patients regarding race, age, initial weight, and duration of use and served as the control group. The 61 patients were selected from a pool of 616 low-risk patients who had been using the same oral contraceptive. All except one of the 61 patients were black.

Table III. The clinical profile of seven patients who discontinued the low-dose oral contraceptive* because of the development of hypertension

No.	Category	*Previous highest blood pressure (mm Hg)*	*Baseline blood pressure (mm Hg)*	*Months of use*	*Blood pressure at discontinuance (mm Hg)*
1	High risk	146/100	130/92	3	155/110
2	High risk	120/90	120/90	4	150/110
3	High risk	140/100	110/80	6	140/102
4	High risk	150/105	120/76	17	148/100
5	High risk	150/94	135/97	25	150/102
6	Low risk	132/82	124/80	3	150/100
7	Low risk	124/80	142/87	21	190/110

*Oral contraceptive containing 0.4 mg of norethindrone and 35 μg of ethinyl estradiol.

Blood pressure readings were determined by one of four paramedical personnel using a mercury sphygmomanometer on the left arm at the cardiac level with the women in a sitting position after at least a 15-minute resting period. The four persons had been trained and had measured blood pressure on all patients in the family planning clinic during the past 9 years. An adult, large blood pressure cuff was used in obese women when a regular cuff would not fit adequately. Systolic pressure was recorded at the first appearance of sound and diastolic pressure at the change of the intensity or quality of sound. When a patient was found to have elevated blood pressure, she was rechecked by a physician who would decide whether the oral contraceptive was to be continued.

A physical examination and laboratory tests including hemoglobin, hematocrit, cervical gonorrhea culture, and Papanicolaou smear were performed annually on all patients. At the initial visit, the patients were counseled regarding various alternatives of contraception and signed an informed consent outlining the risks and benefits of the chosen method.

Two-way analysis of variance of paired observation was used for statistical analysis of blood pressure and body weight measurements. χ^2 Testing was used for comparing the incidence rates of smoking and family history of hypertension between the two groups.

Results

The mean age ± SD of the high-risk group was 24.5 ± 4.2 years which was not statistically different from that in the low-risk group of 23.1 ± 4.0 years.

Seventeen high-risk and eight low-risk patients were smokers. Thirty-seven high-risk and 35 low-risk patients gave a family history of hypertension occurring in parents or siblings. The differences between the two groups were not statistically significant.

In the high-risk group, the highest blood pressures recorded in the past were 141.0 ± 14.9 (mean ± SD) mm Hg systolic and 98.3 ± 8.0 mm Hg diastolic.

Within each group of patients, the mean systolic blood pressure did not change significantly over 24 months of use (Table I), except for lower values recorded at 9 and 18 months, respectively. The baseline systolic blood pressure in the high-risk group was significantly higher than that in the low-risk group. Likewise, systolic blood pressures in the high-risk group during therapy remained significantly higher than those observed in the low-risk group.

Mean diastolic blood pressures within each group during therapy, except at 12 months, were significantly lower than the mean initial blood pressures (Table II). When diastolic blood pressures were contrasted between the two groups, the values before and during oral contraceptive therapy were significantly higher in the high-risk group than in the low-risk group.

Five patients from the high-risk group and two from a pool of 616 low-risk patients discontinued the oral contraceptive because of a rise of blood pressure into the hypertensive range (Table III). Of the five high-risk patients, two had elevated blood pressure in the past associated with oral contraception, and three had elevated blood pressure unrelated to pregnancy or oral contraception. Of the 56 remaining high-risk patients, 40 patients were still using the oral contraceptive without problem, six patients were lost to follow-up, and 10 patients discontinued the contraceptive because of varying reasons such as breakthrough bleeding, headache, or leg cramps. None of the 61 low-risk patients matched with the high-risk group developed hypertension.

There were no significant changes in body weight before or during oral contraceptive therapy either within or between the two groups of patients (Table IV).

Comment

Since race, age, body weight, and a family history of hypertension have been correlated with blood pressure elevation in oral contraceptive users, the matching of

Table IV. Body weights in two groups of women using low-dose oral contraceptive*

	Weight (pounds) (mean ± SD)		
	Low risk	*High risk*	*Probability*
Baseline	151.9 ± 29.8 (61†)	156.7 ± 36.3 (59)	p^r = NS
3 mo	152.2 ± 30.7 (61)	157.1 ± 36.1 (59)	p^t = NS
Baseline	150.2 ± 39.1 (12)	152.9 ± 26.3 (19)	p^r = NS
6 mo	148.4 ± 34.8 (12)	153.7 ± 26.1 (19)	p^t = NS
Baseline	156.3 ± 30.2 (42)	156.6 ± 38.4 (34)	p^r = NS
9 mo	157.1 ± 31.2 (42)	157.9 ± 39.7 (34)	p^t = NS
Baseline	150.3 ± 28.1 (36)	155.7 ± 30.2 (34)	p^r = NS
12 mo	150.2 ± 30.2 (36)	157.8 ± 32.1 (34)	p^t = NS
Baseline	140.5 ± 22.4 (14)	155.3 ± 29.5 (27)	p^r = NS
18 mo	143.3 ± 23.7 (14)	158.3 ± 32.1 (27)	p^t = NS
Baseline	138.2 ± 14.2 (10)	152.8 ± 31.2 (15)	p^r = NS
24 mo	131.6 ± 17.0 (10)	156.6 ± 32.9 (15)	p^t = NS

P^r = Probability with risk as a factor in two-way analysis of variance of paired observations. p^t = Probability with time as a factor in two-way analysis of variance of paired observations.

*Oral contraceptive containing 0.4 mg of norethindrone and 35 μg of ethinyl estradiol.

†Number of patients.

these factors between the two groups of patients would seem to minimize the effect of these variables on blood pressure changes. The observation that the mean body weights in the two groups of patients did not change significantly is consistent with our recent study of the same low-dose oral contraceptive.[11]

That the two groups of patients are distinctly different from each other is supported by the significantly higher blood pressures in the high-risk group both before and during oral contraception than those observed in the low-risk group. Despite these differences, the mean blood pressures in these two groups of women using a low-dose oral contraceptive followed the same patterns in that the mean systolic and diastolic pressures did not rise when compared with their own baseline blood pressures. This is in contradistinction to previous reports[3-6] that users of higher-dose oral contraceptives developed higher mean blood pressures than baseline values. This suggests that the dosage of sex steroids plays an important role in the elevation of mean blood pressures among oral contraceptive users with or without a past history of elevated blood pressure.

The mean diastolic blood pressures in each group during therapy, except at 12 months, were significantly lower than those observed initially. The decrease in mean diastolic blood pressure in the low-risk group is consistent with our recent findings.[11] However, the exclusion of three high-risk patients when hypertension developed before 9 months of therapy could have contributed to lowering of the subsequent mean diastolic blood pressures in the high-risk group.

Although five patients in the high-risk group discontinued the oral contraceptive because of redevelopment of hypertension, the blood pressures at the time of discontinuance were comparable to the highest blood pressures recorded in the past. This gives an incidence of hypertension of 8.2% which is within the range of 0% to 18% observed in other series of higher-dose oral contraceptives in women with few having a past history of elevated blood pressure.[5, 6, 12, 13]

While the pathophysiologic mechanisms for oral contraceptive–induced hypertension and mild elevation of mean blood pressure remain unclear, the renin-angiotensin-aldosterone system is believed to be partially responsible.[14] Plasma renin activity, renin substrate, and angiotensin II concentrations have been found to be elevated during oral contraceptive therapy.[15, 16] In addition, estrogen has been demonstrated to induce sodium retention and to increase both cardiac output and plasma volume.[17, 18] Progestogen doses in excess of 1 mg with the same doses of estrogen have been correlated with the incidence of hypertension in oral contraceptive users.[19] It is, therefore, logical that oral contraceptives with lower dosages of progestogen and estrogen would be associated with a lower or no rise of mean blood pressure and a decreased incidence of hypertension during therapy. While a majority of our patients did not develop hypertension during medication, the occurrence of hypertension after varying periods of therapy in seven patients might be explained on the basis of a diminished feedback suppression of renin release[13] or an increased plasma level of ethinyl estradiol[20] in these patients.

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Management of preterm prematurely ruptured membranes: A prospective randomized comparison of observation versus use of steroids and timed delivery

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The use of hydrocortisone and timed delivery was compared to expectant management of pregnancies complicated by preterm prematurely ruptured membranes in a prospective randomized trial of 73 patients. The incidence of maternal and neonatal complications was compared. There was nc significant difference in the incidence of respiratory distress syndrome. Steroid-treated mothers had an increased incidence of postpartum febrile morbidity. We conclude that treatment of women with preterm prematurely ruptured membranes with hydrocortisone and timed delivery offers no advantage over expectant management. (AM J OBSTET GYNECOL 1985;151:32-8.)

Key words: Prematurely ruptured membranes, steroids, respiratory distress syndrome

Premature delivery and intrauterine infection are the principal fetal risks of preterm premature rupture of the fetal membranes. In an effort to decrease the principal risk of preterm delivery, hyaline membrane disease, without increasing the rupture-to-delivery interval unnecessarily, Mead and Clapp treated women with

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Table I. Entry characteristics of patients in steroid-treated and observation groups

	Steroid-treated group (*n* = *38*)	*Observation group* (*n* = *35*)	*Significance*
Maternal age (yr)	22.4 ± 5.3	24.9 ± 6.9	NS
Parity	1.0 ± 1.1	1.2 ± 1.3	NS
Race			
White (%)	21 (55.3)	24 (68.6)	NS
Nonwhite (%)	17 (44.7)	11 (31.4)	
Private patient (%)	15 (39.5)	18 (51.4)	NS
Clinic patient (%)	23 (60.5)	17 (48.6)	
Week of gestation at admission	31.2 ± 1.6	30.7 ± 1.8	NS
Hours since rupture of membranes at admission	25.7 ± 81.3	19.3 ± 39	NS
Documented immature (%)	20 (52.6)	17 (43.5)	NS
By amniocentesis	12	11	
By vaginal pool phosphatidylglycerol determination	8	6	
Group B streptococcus cultured at admittance (%)	8 (21.1)	6 (17.1)	NS

preterm prematurely ruptured membranes between 28 and 34 weeks' gestation with betamethasone and then delivered the patient 24 to 48 hours after initiation of steroids.[1] These patients, when compared to a nonrandomly selected control group of women managed expectantly, had fewer infants with respiratory distress; there was no difference in the incidence of maternal or neonatal infection. The publication of this study in 1977 led many physicians at The Ohio State University Hospitals to follow the Mead and Clapp protocol of steroids and timed delivery for women with preterm prematurely ruptured membranes. The first 2 years of experience at this hospital with this protocol did not demonstrate any advantage of steroid therapy over expectant management in a retrospective comparison.[2] This prospective trial was therefore designed to enroll and assign women with preterm prematurely ruptured membranes randomly to steroid-treated and observation groups.

Material and methods

The study enrolled patients from September, 1979, through November, 1982. All private, clinic service, and maternal transport patients presenting to The Ohio State University Hospitals with preterm prematurely ruptured membranes at 28 to 34 weeks' gestation were candidates for the study. Preterm prematurely ruptured membranes was documented by either pooled amniotic fluid in the vagina or positive Nitrazine and fern tests. Digital examinations of the cervix were not performed, but some patients had had a single digital examination prior to transfer. Cultures for the group B streptococcus and *Neisseria gonorrhoeae* were obtained from the outer third of the vagina and cervix respectively from all patients at the time of admission. Pooled amniotic fluid in the vagina was sent when possible for phosphatidyl glycerol determination; amniocentesis was performed with ultrasound guidance when an adequate fluid pocket was identified in those patients who did not have pooled fluid in the vagina. Women with mature amniotic fluid lung maturity studies (phosphatidyl glycerol determination positive or lecithin/sphingomyelin ratio of 2:1 or more) were delivered, as were women who manifested labor or evidence of infection (fever, leukocytosis, or bacteria in amniotic fluid obtained by amniocentesis). Patients of <28 or >34 weeks' gestation by best obstetric dating including history, pregnancy milestones, and sonography were not entered into the study. Those of <28 weeks' gestation were managed expectantly; those beyond 34 weeks' were delivered or expectantly managed, but were not considered candidates for steroid treatment and therefore not entered. Twins were excluded as well. The remaining patients were therefore of 28 to 34 weeks' gestation with documented premature amniorrhexis and were neither in labor nor infected. Patients giving informed consent were then randomly assigned by means of a table of random numbers to either the observation or steroid-treatment groups. Those refusing were not further studied.

Those assigned to the observation group were admitted to the antepartum unit, followed with serial vital sign examinations, abdominal examination, and white blood counts. Tocolytic and antipyretic drugs were not used in this group. Delivery occurred after labor, amnionitis, or fetal distress developed. Nine patients in this group were observed without evidence of infection or labor for 7 days and were discharged with instructions to continue bed rest and temperature checks at home. Six of these were later readmitted, but three were eventually delivered elsewhere; adequate data for these three patients and their infants were not available, and so they were excluded.

Patients assigned to the steroid-treated group were given 500 mg of hydrocortisone intravenously every 8 hours for four doses.[3] Tocolytics (magnesium sulfate,

Table II. Maternal outcome

	Steroid-treated group (n = 38)	*Observation group (n = 35)*	*Significance*
Weeks of gestation at delivery	31.4 ± 1.6	31.7 ± 1.9	NS
Hours of ruptured membranes at delivery	89.9 ± 81	125.5 ± 132	NS
Days in hospital	6.1 ± 1.5	6.6 ± 3.3	NS
Cesarean section (%)	8 (21.1)	4 (11.4)	NS
Amnionitis (%)	2 (5.3)	1 (2.9)	NS
Postpartum endometritis (%)	9 (23.7)	2 (5.7)	p = 0.05
Vaginal delivery (%)	4/30 (13.3)	2/31 (6.5)	NS
Cesarean delivery (%)	5/8 (62.5)	0/4	p = 0.08

Table III. Infant outcome

	Steroid-treated group (n = 38)	*Observation group (n = 35)*	*Significance*
Birth weight (gm)	1710.4 ± 457	1753.6 ± 406	NS
Weeks gained—admittance to delivery	0.16 ± 0.4	1.05 ± 1.4	p = 0.001
Apgar score			
1 min	7.2 ± 1.6	6.2 ± 2.4	p = 0.06
5 min	8.4 ± 1.1	8.1 ± 1.1	NS
Perinatal mortality (%)			
Discharged alive	36 (94.7)	33 (94.3)	NS
Transferred	1 (2.6)	1 (2.9)	NS
Died	1 (2.6)	1 (2.9)	NS
Days in hospital	31.4 ± 1.6	31.8 ± 1.9	NS

terbutaline, or ritodrine according to physican preference) were used to delay delivery until 12 hours following completion of steroid therapy when necessary. One patient received only one dose of steroid before delivery despite tocolytic therapy and was excluded. Delivery was effected at 48 to 72 hours after initiation of steroid therapy by discontinuation of tocolysis, induction of labor, or cesarean section.

Indications for cesarean delivery were the same in both groups. All infants in breech presentation were delivered by cesarean section. Prophylactic antibiotics were not given to women who were delivered abdominally. Antepartum antibiotic therapy was used only for patients with clinical amnionitis prior to delivery (temperature 100.6° F and no extragenital source of fever), with culture-proven urinary tract infection, or positive group B streptococcus or gonorrhea cultures. Postpartum endometritis was defined as maternal pyrexia exceeding 100.6° F on two readings 6 or more hours apart exclusive of the first 24 hours post partum with no evidence of an extragenital source of fever. Respiratory distress was diagnosed in newborn infants who required >24 hours of supplemental oxygen and had compatible clinical and chest x-ray findings. Neonatal sepsis was diagnosed when a culture of blood, cerebrospinal fluid, or urine was positive.

Data analysis was performed by χ^2, Fisher's exact test, *t* test, Mann-Whitney U test, and three-way contingency-table log linear analysis for dichotomous outcome data, where appropriate. All probabilities are two-tailed.

Results

Seventy-eight women were enrolled in the study. Five were later excluded from analysis for the following reasons: incomplete data following delivery, elsewhere (n = 3, all in the observation group), major congenital anomaly (n = 1), and failure to complete steroid therapy (n = 1). There were therefore 73 mother-infant pairs available for study, 38 in the steroid-treated group and 35 in the observation group. A comparison of entry characteristics for each group is shown in Table I. The groups appear comparable in maternal demography, duration of pregnancy and of amniorrhexis prior to admission, incidence of immature versus unobtainable pulmonary indices, and vaginal group B streptococcal colonization. The incidence of group B streptococcal colonization for both groups is 19.1%; this compares to an expected 5% to 11% incidence of antenatal group B streptococcal colonization at this hospital.[4] Amniotic fluid for estimation of pulmonary maturity was obtained via transabdominal amniocentesis in 31.6% and 31.4% and by vaginal pooled fluid in 21% and 17.1% of the steroid-treated and observation groups, respectively.

Maternal outcome data are displayed in Table II. There were no significant differences in the duration of pregnancy or amniorrhexis at delivery or in the du-

Table IVA. Length of time from rupture of the membranes to admission

Hours	*Steroid-treated group*	*Observation group*
0-12	26	24
12-24	8	6
24-48	2	2
48	2	3
Total	38	35

Table IVB. Length of time from rupture of the membranes to delivery

	Steroid-treated group			*Observation group*		
Hours	*n*	%	*Cumulative* %	*n*	%	*Cumulative* %
0-24	0	0	0	3	8.6	8.6
24-48	1	2.6	2.6	4	11.4	20.0
48-72	19	50.0	52.6	9	25.7	45.7
72-96	10	26.3	78.9	5	14.3	60.0
96-168	7	18.4	97.4	8	22.9	82.9
≥7 days	1	2.6	100	6	17.1	100

ration of hospital stay. Cesarean section was more common in the steroid-treated group, but the difference did not achieve statistical significance. There were eight cesarean deliveries in the steroid group (four breech, three repeat, and one for suspected fetal distress) and four in the observation group (two repeat and one each for breech and suspected distress). There was no difference in the incidence of antenatal amnionitis, but the steroid-treated group did have significantly more postpartum endometritis. Prophylactic antibiotics for cesarean section were not used in either group. One woman in the steroid-treated group who had not received tocolytics developed mild pulmonary edema which responded promptly to fluid restriction and diuretics. Tocolytic drugs were used in 15 of 38 women in the steroid-treated group. The one patient in whom tocolytic therapy was unsuccessful in delaying delivery was excluded.

Infant outcome data are displayed in Table III. Birth weights were similar as were gestational ages at delivery (see also Table II). Five-minute Apgar scores were not significantly different, but the observation group did have a lower mean Apgar score at 1 minute, perhaps reflecting the lower incidence of abdominal delivery in this group. The perinatal mortality in each group was similar, with only one death in each group. A 920 gm infant born vaginally at 28 weeks' gestation to a steroid-treated mother died of respiratory distress syndrome and intraventricular hemorrhage at 2 days of life. A 1060 gm infant born vaginally 54 hours after membranes ruptured died at 5 days, also of respiratory distress and intraventricular hemorrhage in the observation group. One infant in each group was returned to a referring hospital nursery.

The lengths of time from rupture of the membranes to admission and to delivery are displayed in Tables IVA and IVB. The groups are comparably distributed in duration from rupture to admission but differ in duration to delivery as expected. This is reflected in a significant difference in duration of pregnancy after admission (see Table III).

Neonatal morbidity is shown in Table V. There was no significant difference in the incidence of respiratory distress syndrome or in the number of days of respirator therapy, but those in the steroid-treated group did have a significant decrease in the number of days of supplemental oxygen requirement. Because of the effect of fetal sex upon the incidence of respiratory distress syndrome and possible suceptibility to steroid effect, the incidence of respiratory distress syndrome by sex in each group was further analyzed by three-way log linear analysis for dichotomous outcomes displayed in Table VI. There was no difference between groups in the incidence of respiratory distress syndrome among male infants. A slight decrease in the incidence of respiratory distress syndrome was noted in steroid-treated females, but this did not achieve significance. Females did have less respiratory distress syndrome than males; this achieved statistical significance ($p = 0.037$) in the steroid-treated group but not in the observation group ($p = 0.12$) by Fisher's exact test. Neither the mode of delivery nor the use of tocolytic agents influenced the incidence of respiratory distress syndrome.

There was no difference in the incidence of neonatal sepsis according to the use of steroids antepartum, and there were no deaths caused by sepsis in either group. Relative neonatal leukopenia (white blood cell count $<10,000/mm^3$) was significantly more common in the infants of steroid-treated mothers. Infants of steroid-treated mothers also had lower peak indirect bilirubin values and required significantly fewer days of phototherapy.

Comment

This prospective randomized trial of women with preterm prematurely ruptured membranes shows no significant decrease in neonatal respiratory distress syndrome or mortality when steroid therapy is used, compared to conventional therapy. There was a trend that did not reach significance which suggested that steroid-treated infants required fewer days of ventilator therapy, and these infants did have significantly fewer days of supplemental oxygen therapy. The predominance

Table V. Infant morbidity

	Steroid-treated group ($n = 38$)	*Observation group ($n = 35$)*	*Significance*
Respiratory disease			
Respiratory distress syndrome (%)	10 (26.3)	12 (34.3)	NS
Days on ventilator (mean)	0.63 ± 1.6		NS
Days requiring oxygen (mean)	1.92 ± 2.7	4.97 ± 11.4	p = 0.04
Infectious disease*			
Sepsis (%)	4 (10.5)	3 (8.6)	NS
Leukopenia—white blood cell count <10,000/mm^3 (%)	25 (65.8)	10 (28.6)	p = 0.004
Jaundice			
Peak indirect bilirubin (mean)	3.5 + 4.2	6.6 + 4.3	p = 0.003
Days of phototherapy (mean)	1.1 + 1.5	2.5 + 2.0	p = 0.002

*All infants in both groups were treated with antibiotics.

Table VI. Incidence of respiratory distress syndrome by steroid therapy and sex of infant*

	Incidence of respiratory distress syndrome	
Sex of infant	*Steroid-treated group ($n = 38$)*	*Observation group ($n = 35$)*
Male (%)	7/16 (43.8)	10/24 (41.7)
Female (%)	3/22 (13.6)	2/11 (18.2)

*Three-way log linear analysis for dichotomous outcome data reveals no difference in the incidence of respiratory distress syndrome.

of female infants in the steroid-treated group (22 of 38 were female compared to only 11 of 35 in the observation group) could have skewed the outcome in favor of steroid treatment because of the reported increased susceptibility of females to the respiratory benefits of corticosteroids.[5] The three-dimensional log linear analysis takes this difference in sex distribution into account. Although this analysis confirms the higher incidence of respiratory distress syndrome in males, the sex difference was not related to treatment group. The sample size is not sufficient to deny categorically any neonatal benefit to steroid therapy in women with preterm prematurely ruptured membranes, but is large enough to conclude that any benefit of steroids must be of small magnitude and perhaps is confined to female infants.

Garite et al.[6] in a similarly designed study of 160 patients using betamethasone, found no evidence of benefit in the incidence of respiratory distress syndrome in male or female infants. Seventeen of 79 (21%) infants born to mothers managed expectantly developed respiratory distress syndrome, whereas 14 of 80 (17%) infants born to steroid-treated mothers developed respiratory distress syndrome. The difference is not significant. This study and that of Garite et al. evaluated steroid therapy followed by timed delivery. There are also two papers reporting the outcome of women with preterm prematurely ruptured membranes randomly assigned in blind fashion to placebo or steroid therapy followed by expectant management. Both were reported as part of larger studies of steroids in preterm gestation. The first by Tauesch et al.[7] in 1979 reported on 81 women with preterm prematurely ruptured membranes. Seven of 34 infants (20.6%) born to mothers who received dexamethasone developed respiratory distress syndrome, whereas 14 of 47 (29.8%) born to placebo-treated mothers had respiratory distress syndrome. This difference is not statistically significant ($\chi^2 = 1.01$, $p = 0.3$), although there is a trend in favor of steroid treatment. The Collaborative Group on Antenatal Steroid Therapy reported 288 cases of preterm prematurely ruptured membranes randomly assigned to receive either dexamethasone or placebo and followed expectantly.[8] Fifteen of 153 infants whose mothers received steroids (9.8%) developed respiratory distress syndrome, compared to 17 of 135 (12.6%) infants who developed respiratory distress syndrome in the placebo group. This difference is also not statistically significant ($\chi^2 = 0.57$, $p = 0.7$). Whether the endogenous stress of preterm amnion rupture mimics and therefore obliterates the therapeutic effect of exogenous steroids seen in patients with intact membranes remains uncertain.[9]

Garite et al.[6] and Tauesch et al.[7] both reported an increased incidence of postpartum infection in steroid-treated mothers: 11% in expectantly managed versus 29% of steroid-treated mothers in Garite's study, and 12% of expectantly managed versus 27% of steroid-treated mothers in Tauesch's report. Our data is similar, with 5.7% and 23.7% postpartum endometritis rates for observation and steroid-treated groups respectively. Garite's patients who were delivered abdominally all received prophylactic antibiotics; the incidence of endometritis in observed and steroid-treated mothers delivered by section was similar, but there was a pronounced increase in the incidence of endometritis in Garite's steroid-treated mothers delivered vaginally ($p = 0.025$) who did not routinely receive prophylactic antibiotics. In our study, the increase in postpartum endometritis among steroid-treated patients is most likely due to the high (5/8) incidence of infection following cesarean section in steroid-treated patients. This apparent effect on the incidence of postpartum febrile morbidity may be due to a steroid-mediated alteration in host response but could also be explained by the greater number of cesarean sections in the steroid group or by a difference in one of the other standard risk factors for puerperal infection such as duration of labor, number of vaginal examinations, maternal weight, operative time, anesthesia mode, or duration of indwelling intrauterine catheter placement, factors for which we did not control.

There are several other findings which deserve comment. Assuming one accepts our findings and those of Garite et al., Tauesch et al., and the Collaborative Group cited earlier and abandons the use of steroids in women with preterm prematurely ruptured membranes, the management plan for each patient will be determined by an individual assessment of the risks of delivery (prematurity) versus the risks of expectant management (infection and prolapsed cord). Assessment of fetal lung maturity was accomplished in about half of our patients; an unrecorded number of women with preterm prematurely ruptured membranes had amniotic fluid studies indicating fetal lung maturity and were not enrolled. The authors and house staff participating in the study were often surprised that so many women of apparently <34 weeks' gestation had fluid studies indicating maturity. Amniotic fluid is often available from women with preterm prematurely ruptured membranes and provides a rational basis for interventionist or expectant management.[10] Means of estimating the risk of infection for expectantly managed patients with preterm prematurely ruptured membranes are less secure, but it is again important to look for known pathogens such as the group B streptococcus when expectant management is chosen. Six of 35 women expectantly managed were colonized at the time of admission with group B streptococcus. All were treated with oral amoxicillin or ampicillin and recultured when possible. Nevertheless, three of these infants were colonized at birth, and one had documented group B streptococcal sepsis from which he recovered. Thus intravenous rather than oral ampicillin may be necessary in these patients.[11] One could argue that a positive vaginal group B streptococcus culture at the time of admission should lead first to intravenous ampicillin followed by termination of expectant management. The number of cases here is too small to draw conclusions, other than to state that expectant management in the face of a positive group B streptococcus culture is problematic. Perhaps in the future it will be possible to assess the antibacterial activity of amniotic fluid from women with preterm prematurely ruptured membranes in order to further quantify their likelihood of infection. That approach has been frustrating to date.[12]

The success in the steroid-treated group in delaying delivery, with only one patient lost due to incomplete steroid therapy, is probably due to a self-selection process at the time of entry. Patients thought to be in labor were not candidates for enrollment, and so a period of observation for possible uterine acvitity while amniotic fluid studies were pending was often the case before enrollment and subsequent randomization. We felt this was advisable in order to avoid the maternal risk of aggressive tocolysis in steroid-treated patients and the potential long-term risk of steroids without a chance for short-term benefit.

The rate of spontaneous labor in the observation group was consistent with the experience of others.[9] Fifty percent of women with preterm prematurely ruptured membranes who were managed expectantly delivered spontaneously in the first 3 days and 83% within the first 7 days. We feel that "anticipatory" transport of women with preterm prematurely ruptured membranes to a perinatal center is therefore advisable to gain the documented advantage of preterm delivery in a high-risk center.[13] For those 20% of women who remain undelivered and uninfected 1 week after amniorrhexis, management can then be individualized.

Two unexpected findings in the infants of steroid-treated mothers were the increased (65%) incidence of relative leukopenia (white blood count $<10{,}000/mm^3$), and the significant decrease in the mean indirect bilirubin and concomitant decrease in the duration of phototherapy in this group. The decreased white blood counts may be related to the slightly higher rate of abdominal delivery in the steroid-treated group, sparing the fetus the physiologic stress of labor and corresponding rise in white blood counts. It is tempting to speculate that maternal steroid treatment accelerates maturation of fetal hepatic glucuronyl transferase activity, which would explain the diminished neonatal jaundice. Many other factors such as mode of delivery, incidence of traumatic delivery and hematoma formation, and hematocrit would need to be controlled before accepting such a conclusion from these data.

In summary, we believe that this series, when considered with others in the literature, provides additional evidence that any fetal benefit of antenatal steroid therapy in women with prematurely ruptured membranes between 28 and 34 weeks' gestation is of small magnitude. Given the overall excellent outcome of expectantly managed pregnancies and the possibility of increased infectious morbidity in steroid-treated mothers, steroid therapy appears to have no advantage over expectant management in these women. Researchers should look elsewhere to improve the outcome of these pregnancies.

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Antenatal diagnosis of renal anomalies with ultrasound

III. Bilateral renal agenesis

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Bilateral renal agenesis is a lethal congenital anomaly. A reliable prenatal diagnosis is extremely important, since it may offer options for pregnancy termination or may change obstetric management in the third trimester. This study examined the accuracy of ultrasound in making an antenatal diagnosis of bilateral renal agenesis in three different populations: (1) patients with a family history of bilateral renal agenesis, (2) patients diagnosed during the course of a routine scan, and (3) patients referred because of a previous suspicious ultrasound examination in a level I ultrasound facility. In group A there were three true positive, 13 true negative, no false negative, and no false positive diagnoses. In group B there were three true positive and no false positive diagnoses. In group C there were 12 true positive, 17 true negative, one false negative, and no false positive diagnoses. The value and potential pitfall of the different diagnostic criteria are discussed. We conclude that ultrasound is a valuable tool in the detection of intrauterine renal failure, although there are limitations to a specific diagnosis of bilateral renal agenesis. (AM J OBSTET GYNECOL 1985;151:38-43.)

Key words: Renal anomalies, ultrasound, bilateral renal agenesis

Bilateral renal agenesis is a lethal congenital anomaly with an incidence of 0.3 of 1000 births.[1] The term Potter syndrome refers to the combination of bilateral renal agenesis, pulmonary hypoplasia, typical facies, and aberrant hand and foot positioning.[2, 3] Isolated case reports have documented the feasibility of making

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Table I. Diagnostic accuracy of ultrasound in the antenatal diagnosis of bilateral renal agenesis

Study group	*No. of cases*	*Diagnosis*			
		True positive	*True negative*	*False positive*	*False negative*
Family history of bilateral renal agenesis	16	3*	13	0	0
Incidental finding on routine scan	4649	3	†	0	†
Previous suspicious scan in Level I facility	30	12	17	0	1

*In one case the diagnosis was delayed until the third trimester.

†Precise figures not available as not all perinatal deaths underwent autopsy.

an antenatal diagnosis with sonography.[4-7] However, the diagnostic accuracy of this technique has not been examined in a large series. A reliable prenatal diagnosis of this uniformly lethal condition would be extremely important as it may offer options for elective termination of pregnancy prior to viability or may change obstetric management in the third trimester. The purpose of this communication is to examine the accuracy and limitations of sonography in the antenatal diagnosis of bilateral renal agenesis.

Material and methods

From January 1, 1978, to December 31, 1982, 18 fetuses were diagnosed at the perinatal ultrasound unit of Yale–New Haven Hospital as having Potter syndrome. These cases emanated from three different populations. Group A consisted of 16 patients referred to our unit because of a history of having previously been delivered of an infant with Potter syndrome. Group B consisted of 4649 patients who were scanned in our unit and delivered at Yale–New Haven Hospital. Ultrasound examination was requested in these patients because of obstetric indications (discrepancy between sizes and dates, dating, placental location, etc.). Group C consisted of 30 patients referred from level I ultrasound facilities with the suspicion of a renal anomaly suggested by oligohydramnios and uncertainty about the visualization of the fetal bladder or the presence of kidneys. In all patients with a positive antenatal diagnosis, autopsy reports were available. Neonatal follow-up was available for all infants referred because of a positive family history (group A) as well as those referred from level I ultrasound facilities with a suspicion of a renal anomaly (group C). The birth prevalence of Potter syndrome in infants delivered at Yale–New Haven Hospital during the study period was determined from a review of the autopsy report of all perinatal deaths.

Sonographic examinations were performed using a static B scanner with a 3.5 MHz transducer (Picker, North Haven, Connecticut) and/or linear-array real-time equipment with a 3.5 MHz transducer (General Electric, Milwaukee, Wisconsin; ADR, Tempe, Arizona; Picker, North Haven, Connecticut).

The antenatal sonographic diagnosis of renal agenesis was based on the combination of severe oligohydramnios, absence of fetal bladder, and failure to identify fetal kidneys.

Results

Table I demonstrates the diagnostic accuracy of ultrasonography in the antenatal diagnosis of bilateral renal agenesis. Of the 16 patients with a family history of the anomaly (group A), there were three true positive and 13 true negative diagnoses. Three true positives occurred in the 4649 patients who had antenatal sonograms for obstetric indications and subsequently delivered at our institution (group B). No false positive diagnosis was found in this group. The precise incidence of false negatives cannot be accurately calculated, since not all stillbirths or neonatal deaths had autopsies.

Of the 30 patients referred from level I ultrasound facilities with the suspicion of renal anomalies (group C), there were 12 true positive, one false negative, no false positive, and 17 true negative diagnoses. Of the 17 true negative diagnoses, there were 12 infants without congenital anomalies, two with dysplastic kidneys, one with infantile polycystic kidney disease, one with a multicystic kidney and hydrocephaly, and one with a multicystic left kidney and absence of the right kidney. Antenatal identification of the specific renal anomaly occurred in all cases except for the unilateral renal agenesis.

The one false negative diagnosis occurred in a patient referred for discrepancy of size and dates at 36 weeks gestation. Although oligohydramnios and a nonvisualized bladder suggested bilateral renal agencies, we were uncertain about the absence of kidneys. In view of the presence of massive hydrocephaly and severe renal dysfunction, the decision was made against aggressive intervention for fetal indications.

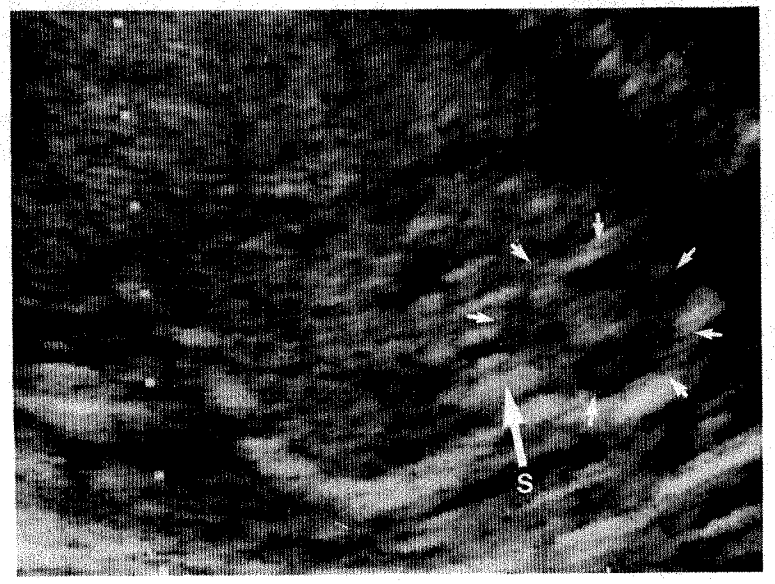

Fig. 1. Transverse scan of the fetal abdomen in Case 19. The paraspinal image indicated by the *arrows* corresponds to an adrenal gland in a fetus with bilateral renal agenesis.

Of the 18 correctly diagnosed cases there were seven fetuses diagnosed prior to 24 weeks' gestation. These patients included two from the group with a family history of bilateral renal agenesis, two referred from a level I ultrasound facility because of the suspicion of renal anomaly, and the three remaining who underwent sonography because of exposure to progesterone in early pregnancy (1 case), elevated maternal serum α-fetoprotein (1 case), and midtrimester amniocentesis because of maternal age >35 years (1 case). All the patients with a family history of bilateral renal agenesis were scanned prior to 24 weeks' gestation. In one case the diagnosis was made after 24 weeks' gestation. Her scan, at 21 weeks, demonstrated oligohydramnios, and there was uncertainty as to the absolute absence of a fetal bladder. Fetal kidneys were thought to be present. At a later scan (25 weeks' gestation) no bladder was visualized and the paraspinal images were again thought to correspond to kidneys. However, after delivery in the previous false negative case, the films were reviewed and the similarity between the lumbar fossae in both cases was striking and the diagnosis of renal agenesis was made prior to delivery. Nonaggressive intervention for fetal indications was advised to the referral physician. Of the 30 patients referred from level I ultrasound facilities with the suspicion of a renal anomaly, 16 were referred prior to 24 weeks. All these patients had been scanned in a level I facility because of size and dates discrepancies. The precise number of patients scanned before 24 weeks of gestation for obstetric or genetic indication (group B) in our unit over the 5-year studied period was not determined.

Sonographic findings were as follows: of the 19 affected fetuses, a bladder was not visualized in 18, oligohydramnios was present in all, and fetal kidneys were finally thought to be absent in 18 cases.

Comment

In bilateral renal agenesis, as in any other uniformly lethal disorder, a reliable prenatal diagnosis is extremely important. False positive diagnoses could result in elective terminations of pregnancy or in suboptimal management of viable unaffected infants. False negative diagnosis could result in an unnecessary cesarean section, not infrequently performed because of intrapartum fetal distress or malpresentations in infants with bilateral renal agenesis.[8]

This report addresses the diagnostic accuracy of sonography in three different populations: (1) patients with a history of having delivered a child with bilateral renal agenesis, (2) patients referred to our ultrasound unit because of routine obstetric or genetic indications (e.g., elevated maternal serum α-fetoprotein, midtrimester amniocentesis because of maternal age), and (3) patients referred from level I ultrasound facilities with the suspicion of bilateral renal agenesis or a severe renal anomaly.

The first population examines the ability of ultrasound to identify the disease in a population at risk. Bilateral renal agenesis seems to be transmitted in a polygenic pattern with a suggested recurrence rate of 2% to 5%. Some cases with a possible autosomal recessive and X-linked inheritance have also been reported.[9, 10] The correct antenatal diagnosis was made in

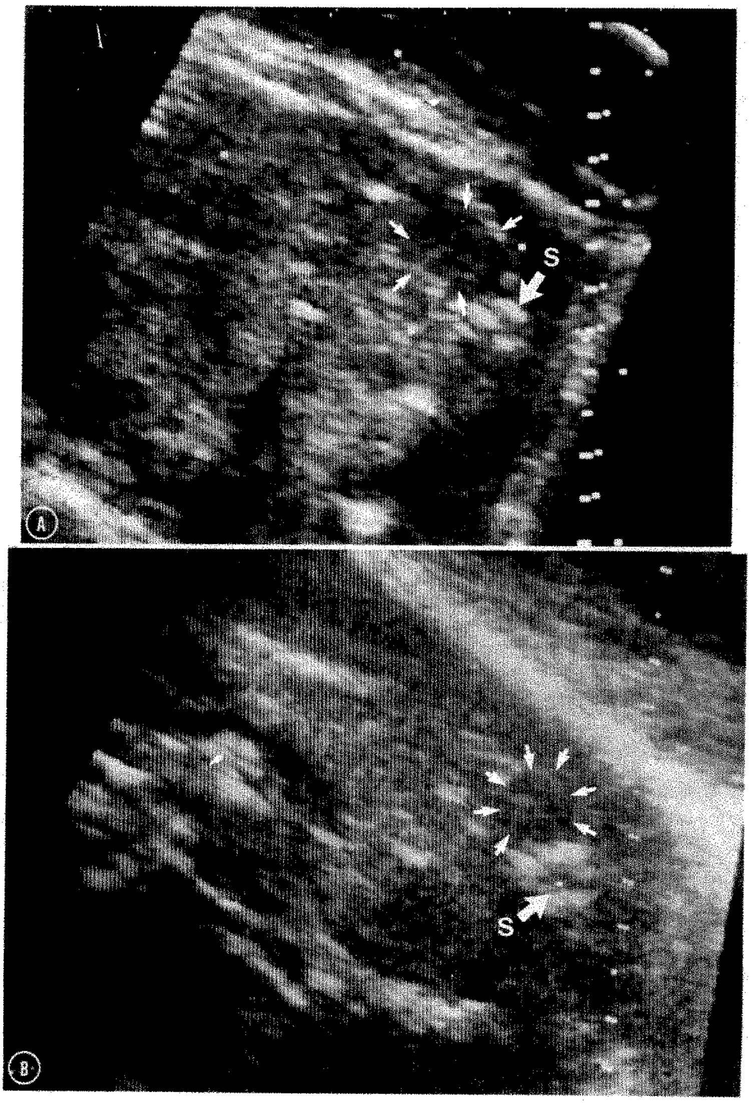

Fig. 2. *A*, Transverse scan of the abdomen of Case 2 at 21 weeks' gestation. *Small arrows* outline enlarged fetal adrenals originally thought to correspond to fetal kidneys. *B*, Transverse scan of the fetal abdomen in the same patient at 26 weeks' gestation. The *arrows* outline a paraspinal mass without a capsule or a clearly visualized renal pelvis. *S*, Spine.

the three affected infants and excluded in the remaining 13. In two cases the diagnosis was made prior to 24 weeks' gestation based on the presence of oligohydramnios, a nonvisualized fetal bladder, and the absence of kidneys. In one case, the diagnosis was delayed because of initial uncertainty about nonvisualization of a fetal bladder and the presence of kidneys. At a later time the bladder was not visualized in repeated scans and it was realized that the retroperitoneal paraspinal masses represented adrenal glands. This was later confirmed at autopsy when the adrenal glands were found to be enlarged and weighed 10 gm (left) and 10.5 gm (right) (normal combined fetal adrenal weight at 39 weeks' gestation is 8.5 gm[11]). This potential source of confusion has been previously recognized in both fetuses and neonates.[7, 12] Potter[13] has suggested that the adrenal glands in bilateral renal agenesis adopt an oval disk shape presumably because of the absence of a compressive effect by the normal kidney. This shape can grossly simulate the morphology of a normal fetal kidney (Figs. 1 to 3). It is possible that with the improved knowledge of both intrarenal and adrenal anatomy this potential source of error can be minimized in the future. Criteria that may be helpful in distinguishing between the two organs include clear identification of the renal capsule and consistent imaging of the renal pelvis.

The second population (group B) addresses the ability of experienced ultrasonographers to detect bilateral renal agenesis in patients referred for examination be-

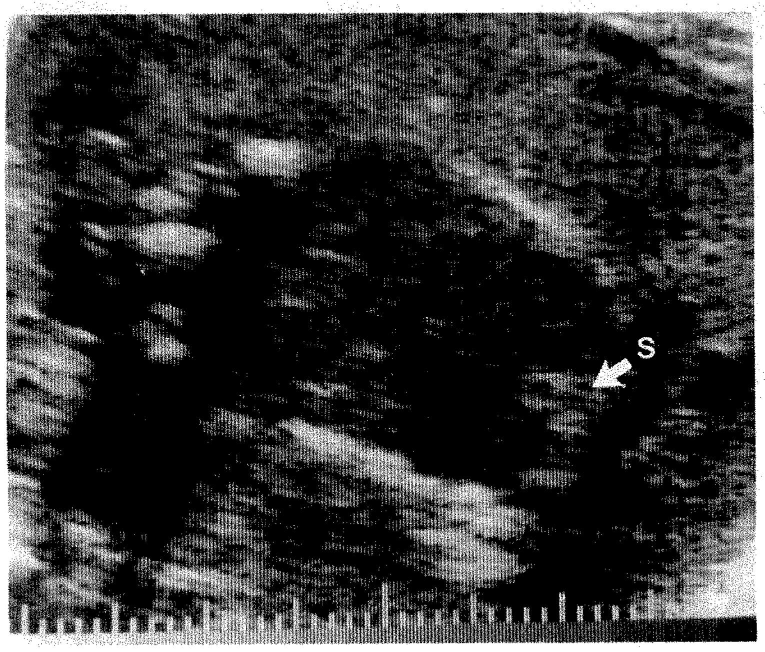

Fig. 3. Typical image of the renal fossae in renal agenesis. *S*, Spine.

Table II. Clinically assigned causes of perinatal death in infants without autopsies during the studied period

Cause of death	*No. of cases*
Extreme prematurity	185
Congenital heart disease	16
Necrotizing enterocolitis	6
Anencephaly	4
Intraventricular hemorrhage	4
Meconium aspiration syndrome	3
Burns	2
Perinatal anoxia	5
Multiple congenital anomalies*	2

*Bilateral renal agenesis excluded by postnatal sonography.

cause of obstetric or genetic indications. Three true positive diagnoses occurred in this group. No false positive diagnosis was made. As previously stated the number of false and true negative diagnoses could not be determined with absolute accuracy. The precise incidence of false negative diagnosis cannot be accurately calculated, since not all stillbirths or neonatal deaths had autopsies. However, a reasonable estimate of the likelihood of a missed diagnosis of bilateral renal agenesis can be obtained from the following calculation: over the studied period there were 676 perinatal deaths (457 neonatal and 219 stillbirths). The total number of autopsies of this group was 365 (53%). Of the autopsied infants, 9 had bilateral renal agenesis. Six of these infants were antenatally diagnosed (one from Group A, two from group B, and three from group C), and the remaining three did not have prenatal sonography. Of the 311 patients that did not have autopsies, 232 had an identifiable cause of death (Table II). This leaves 79 (8.1%) unexplained perinatal deaths, in which all fetuses underwent a gross examination by a pathologist and none had stigmas of Potter syndrome. It is therefore unlikely that cases of bilateral renal agenesis were missed.

Group C illustrates the value of a level II ultrasound facility as a consulting service for level I ultrasound units. There were no false positive and one false negative diagnoses. Ultrasound demonstrated reasonable diagnostic accuracy in this high-risk population with a prevalence of bilateral renal agenesis of 40%. Importantly, the diagnosis was excluded in 12 patients who ultimately were delivered of infants without congenital anomalies.

The proposed diagnostic criteria for bilateral renal agenesis include the following: oligohydramnios, nonvisualization of a fetal bladder, and absence of kidneys. Oligohydramnios is thought to result from the lack of production of fetal urine, an important component of amniotic fluid. However, it has been suggested that prior to 16 weeks' gestation the contribution of fetal urine to amniotic fluid volume is minor[14] and therefore it is possible that oligohydramnios is not invariably present in bilateral renal agenesis in the early midtrimester. Furthermore, cases of bilateral renal agenesis without oligohydramnios have been reported in association with other defects that impaired the disposition of amniotic fluid (e.g., esophageal atresia, severe central nervous system defects that impair the neural control of deglutition).[3, 15]

Although the absence of fetal kidneys should be the most specific finding in bilateral renal agenesis, the previously discussed potential confusion with enlarged or abnormally shaped adrenal glands must be recognized. More observations are necessary to establish whether the improved resolution of ultrasound equipment and the knowledge of the intrarenal and adrenal anatomy will permit reliable distinction of these two organs in the presence of oligohydramnios.

It seems that repeated and consistent nonvisualization of a fetal bladder is a reliable sign of in utero failure to produce urine (in the absence of urinary obstruction). A fetal bladder is easy to identify and should be demonstrated in every sonographic examination. Its absence after 16 weeks of gestation should prompt further investigation. It can result from recent fetal micturition or be a sign of renal failure. Reexamination of the patient should solve this problem. In cases of doubt, furosemide can be administered to the mother, since this drug will cross the placenta and induce fetal diuresis.[16] Failure to identify a fetal bladder will suggest urinary failure. Examination of the kidneys should then be performed to establish the cause of the problem. Hyperechogenic enlarged kidneys would suggest infantile polycystic kidney disease, and absent kidneys suggest bilateral renal agenesis.

Addendum

Since the submission of this manuscript, we have had three cases in which a false positive diagnosis of renal agenesis was made. These fetuses had a nonvisualized bladder and severe oligohydramnios and failed to have a furosemide-induced diuresis. At the time of sonography they were thought not to have kidneys. After delivery, kidneys were present and were morphologically normal in two and abnormal in one. These observations have made us reexamine our position regarding a confident diagnosis of bilateral renal agenesis. We believe that ultrasound may detect intrauterine renal failure associated with absent or functionally abnormal kidneys but believe that in some cases of pre–renal abnormality (intrauterine growth retardation?) it may be impossible with the present resolution of ultrasound equipment to establish unequivocally the absence of kidneys. Our experience opens questions about the reliability of furosemide-induced diuresis to differentiate between renal agenesis and other cases of intrauterine renal failure. Options such as the instillation of fluid in the amniotic cavity may help improve the specificity of ultrasound in the diagnosis of bilateral renal agenesis.

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Prenatal detection of congenital malformations by ultrasonography

Mayo Clinic experience

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Routine obstetric ultrasonography has been performed at our institution since 1974. During a recent 41-month period, 64 neonates with 83 congenital abnormalities had been scanned before delivery. The detection rate according to the organ system involved and the year in which the study was performed is reviewed. Suggestions, based upon the data presented, are given for the various anatomic sites and fetal dimensions that should be evaluated during each stage II ultrasound examination. (AM J OBSTET GYNECOL 1985;151:44-50.)

Key words: Ultrasound, congenital, malformations

The improved resolution obtained with current ultrasound equipment has permitted sonographers to delineate normal fetal anatomy in increasingly finer detail.[1] Detection of abnormal growth or of a failure in the normal developmental processes has become possible through the use of ultrasound. With this information, congenital abnormalities can be either detected or suspected at a relatively early gestational age. Depending on the specific abnormality, second-trimester patients can be offered a stage II ultrasound examination in an attempt to exclude the recurrence of a known inherited defect. The diagnosis of a specific congenital abnormality during an ultrasound examination in the third trimester can affect not only the obstetric management but also the early neonatal care. Hence, it is important for the obstetrician to be cognizant of both the potential and the limitations of ultrasonography for detection and correct diagnosis of congenital abnormalities.

This report reviews our current experience with the ultrasonic assessment of fetal malformations.

Material and methods

The Mayo Clinic is a level 3 referral center for southeastern Minnesota, northwestern Iowa, and southwestern Wisconsin. Since 1974, routine obstetric ultrasonography has been performed in the last part of the second or early part of the third trimester.

Over a 41-month period (October, 1979, through February, 1983), a total of 9230 sonograms had been obtained on the 5420 patients who were delivered: a single ultrasound examination was performed in 58.1%, two scans in 29.4%, three scans in 8.7%, and four or more scans in 3.8%. Each patient was scanned in a semirecumbent position. The intrauterine environment was initially evaluated by means of a linear array real-time system* with a 3.5 MHz transducer. The fetal lie, the gestational age assessment, the semiquantitative estimate of amniotic fluid volume, and the placental location and grade were determined. A cursory systematic fetal organ review was then performed in an attempt to detect any gross congenital abnormalities. A B-mode gray-scale scanner† was then used to obtain an overview of the intrauterine environment in both the longitudinal plane and the transverse plane.

During the study period, members of the Department of Pediatrics discovered 83 congenital anomalies in 64 neonates within the first month of life. In every instance, either the sonographic diagnosis was confirmed or there were anomalies that had not been detected at the time of the ultrasound examination. Although there were no false positive diagnoses, several patients were evaluated with serial ultrasound examinations until the sonographer was convinced that variations in fetal anatomy or organ size were within the acceptable limits of normal.

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*Picker Model LS-2000 linear scanner, Picker Corporation, 12 Clintonville Road, Northford, CT 06472.

†80-L digital scanner, Picker Corporation.

Table I. Congenital malformations: Central nervous system

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Anencephaly	4	4	4	0.75/1000	2
Hydrocephalus	2	2	2	0.75/1000	2
Encephalocele	1	1	1	0.14/1000	2
Hydranencephaly	2	2	1	Rare	
Holoprosencephaly	1	1	0	1/6000 (genetic, familial, idiopathic)	3
Microcephaly	1	0	0	0.19/1000	2

Table II. Congenital malformations: Orogastrointestinal tract

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Cleft lip or palate	5	0	0	1/1063	11
Imperforate anus	2	0	0	1/5000	11
Tracheoesophageal fistula	2	0	0	1/1000	3
Omphalocele	2	0	0	1.4/10,000	2
Duodenal atresia	1	1	1	1/10,000	11
Gastroschisis	1	1	1	1/20,000	11
Diaphragmatic hernia	1	1*	0	1/5000	12
Duplication of terminal ileum	1	0	0	Rare	3

*In one infant with multiple congenital anomalies, the diaphragmatic hernia was not detected.

Anatomic anomalies

Central nervous system. Each of the 10 malformations of the central nervous system that occurred during the study period (Table I) was diagnosed or suspected antenatally. The neonate with congenital microcephaly had been ultrasonically normal at the gestational age when scanning was performed.

The earlier the diagnosis of hydrocephaly is made, the easier it is to differentiate normal cerebral anatomy. An increase in lateral ventricular dilatation with time helps confirm the diagnosis.[4] Once massive ventricular dilatation occurs, however, compression of normal brain architecture can resemble destructive lesions of the brain (hydranencephaly, porencephaly). Because it is now technically possible to place a ventriculoamniotic shunt in a premature fetus with hydrocephalus,[5] the antenatal identification of specific pathologic entities in the central nervous system is crucial. In addition, the obstetric management can vary from transabdominal encephalocentesis and vaginal delivery[6] to premature delivery by cesarean section (after lung maturation has been confirmed) followed by neonatal ventriculoperitoneal shunting. One of the two cases of hydranencephaly in this study was originally diagnosed as hydrocephalus and, as a result, cesarean section was performed rather than a cranial decompression and vaginal delivery. Occasionally, antenatal computed tomography can be of assistance in the confirmation of a diagnosis that is suspected from ultrasonic findings.[7]

In the only case of microcephaly in this series, scanning was performed at 14 and 22 weeks. The biparietal diameter, cephalic index,[8] and head circumference[9] were within normal limits at those times. Most cases of microcephaly, however, are not detected until the third trimester. Kurtz et al.[10] divided microcephaly into three groups on the basis of the antenatal sonographic findings: (1) true microcephaly (biparietal diameter >3 SD below the mean) with associated mental retardation, (2) relative microcephaly (biparietal diameter 1 to 3 SD below the mean), and (3) pseudomicrocephaly (a small biparietal diameter because of an abnormally shaped head).

Gastrointestinal system. The 15 congenital abnormalities of the orogastrointestinal tract encountered during the study are outlined in Table II. Three of the fetuses had abnormal sonograms, and two of the abnormalities were diagnosed antenatally.

Antenatal ultrasonic detection of cleft lip or palate has been reported.[13] In our series, however, none of the mothers had previously given birth to a child with this defect. Therefore, the anomaly was not specifically looked for, nor was it detected on routine ultrasonography.

A sonogram obtained at 22 weeks' gestation for assessment of gestational age failed to reveal a large omphalocele containing liver and small and large bowel. A much smaller omphalocele was likewise undetected on a routine scan at 26 weeks' gestation.

The single case of gastroschisis was detected at 32

Table III. Congenital malformations: Cardiothoracic system

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Cardiac (congenital heart disease)	18	3	3	6/1000	15
Atrial flutter with variable ventricular response	1	1	1	Arrhythmias, 0.05/1000	2
Supraventricular tachycardia	1	1	1		
Premature atrial contractions, premature atrial tachycardia	1	1	1		
Common atrium, atrial septal defect	2	0	0	1/1000	3
Transposition of great vessels	2	0	0	1/2000	3
Ventricular septal defect	3	0	0	1/400	3
Pulmonary valve atresia	1	0	0	1/10,000	3
Hypoplastic pulmonary artery	2	0	0	1/1250	3
Mitral valve atresia	2	0	0	2.9/10,000	16
Patent ductus arteriosus	1	0	0	1/2000	3
Aortic stenosis	1	0	0	1/2000	3
Hypoplastic left heart	1	0	0	3.65/10,000	16
Thoracic	3	1	1		
Asphyxiating thoracic dystrophy	1	0	0	Rare	
Cystic hygroma	1	1	1	0.04/1000	2
Laryngeal hypoplasia	1	0	0	0.05/1000	2

Table IV. Congenital malformations: Genitourinary system

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Urethral atresia or stenosis	2	2	1	1/10,000	18
Bilateral renal agenesis	3	3	1	1/4000	19
Posterior urethral valves	2	2	1	1/1000 (neonate, child, adult); 1/25,000 (severe obstruction)	20 21
Cystic disease of the kidney	1	1	1	2-4/1000	3
Obstruction at ureteropelvic junction	2	2	2	1/1250	18
Prune-belly syndrome	2	2	1	1/40,000	22
Solitary kidney	1	0	0	1/1500	11

weeks' gestation. After delivery by cesarean section, a primary repair of the defect was performed without difficulty. The diagnosis of duodenal atresia was made on routine scanning at 29 weeks' gestation. This infant also had Down's syndrome.

Mothers of both infants with a final diagnosis of imperforate anus had undergone scanning before 25 weeks' gestation, which is before the fetus begins to ingest a substantial amount of amniotic fluid. As a result, the sonolucent pattern characteristic of distal colonic obstruction was not perceptible.[14]

Neither a tracheoesophageal fistula nor duplication of the terminal ileum has been detected ultrasonically, and these are mentioned merely for completeness.

Cardiothoracic system. Nine neonates with either congenital heart disease or a rhythm disturbance were delivered. Only the three cardiac arrhythmias were diagnosed antenatally (Table III). One infant with supraventricular tachycardia and secondary congestive heart failure was successfully treated by administration of digoxin and propranolol to the mother.[17]

Fetal cardiac scanning to rule out congenital anomalies was not routinely performed during the years of this review; therefore, congenital heart disease in six neonates went undetected until after delivery. None of these infants was small for gestational age at delivery (birth weights were between 2,740 and 3,750 gm).

The single case of asphyxiating thoracic dystrophy was extremely mild; this infant was developing normally at 11 months of age. In another case, detection of a massive cystic hygroma at 28 weeks led to the vaginal breech delivery of a nonviable infant.

Genitourinary system. All 12 malformations of the genitourinary system were either diagnosed antenatally (seven) or were associated with an abnormal ultrasound examination (five). In the latter instances, a specific diagnosis could not be made antenatally. One case of caudal regression syndrome and a solitary kidney went undetected (Table IV).

Various degrees of urethral obstruction were distinguished by the effect of the blockage on amniotic fluid volume and by the condition of the more proximal

Table V. Congenital malformations: Skeletal system

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Osteogenesis imperfecta	3	3	1	1/20,000	2
Craniosynostosis	1	1	0	2.3/10,000	2
Bilateral congenital absence of fibula	1	0	0	Rare	3
Caudal dysplasia syndrome	2	0	0	1/200,000 (overall); 1/1000 (diabetic infants)	3
Femoral hypoplasia	1	0	0		
Bilateral absence of first metacarpals	1	0	0	1/200,000	3
Absence of digits 2, 3, and 4 of the hand	1	0	0	1/200,000	3

Table VI. Congenital malformations: Chromosomal anomalies

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Trisomy 21	6	0	0	1/770	3
Trisomy 18	1	0	0	1/6600	3
Triploidy (69,XXY)	1	1	0	1/2500	28
Trisomy 1 (q32→qter)	1	0	0	Rare	

renal system. Megalocystis and oligohydramnios were present at 16 weeks' gestation in a fetus with urethral atresia.

Multiple loops of hydroureters were visualized in two fetuses with prune-belly (Eagle-Barrett) syndrome due to posterior urethral valves. Neither infant survived the neonatal period. The cystic kidneys characteristic of renal dysplasia were seen ultrasonically in one fetus with posterior urethral valves.

Complete obstruction at the ureteropelvic junction was easily diagnosed ultrasonically on a routine scan at 29 weeks' gestation.

In the only case of primary renal dysplasia in our series, renal function was sufficient to permit visualization of the urinary bladder. Although there was a subjective decrease in amniotic fluid volume, severe oligohydramnios (less than a 1 cm pocket of amniotic fluid) was not present.

Recently, attempts have been made to reduce the deleterious effect of urinary tract obstruction on subsequent renal development by placing bladder-amniotic shunts in selected cases. Strict criteria must be used in the selection of fetal candidates for the procedure so that unnecessary or possibly traumatic intervention is not attempted.[23, 24]

Skeletal system. The nine skeletal abnormalities detected during the study in general were rare ones (Table V). Only one was diagnosed antenatally. Ultrasound examination revealed abnormalities in three patients with osteogenesis imperfecta. Sonographic findings consistent with significant growth retardation were obtained in one of these three. In the two other patients, the following ultrasonic features that were present were consistent with osteogenesis imperfecta: evidence of bone demineralization, head:abdomen ratio above the ninety-fifth percentile, deformed fetal calvarium with unusual clarity of the cerebral gyri, and shortened limb bones. In addition, an outline of the fetal skeleton could not be seen on an abdominal roentgenogram.[25, 26]

Craniosynostosis causes various deformities of the skull, depending on which sutures close prematurely. A sonogram showed the calvarium to be long and narrow in the only cases of this abnormality in our series. The biparietal diameter was 6 weeks behind the stated gestational age of 38 weeks. Although craniosynostosis was not suspected at the time of scanning, the application of current obstetric techniques (cephalic index,[8] head circumference,[9] and femur:biparietal diameter ratio[27]) might have led one to suspect the correct diagnosis.

Neither woman who gave birth to an infant with caudal dysplasia syndrome was diabetic. The diagnosis was not suspected in either instance after a routine ultrasound examination. Bilateral femoral hypoplasia was an associated anomaly in one infant with caudal dysplasia.

Chromosomal anomalies

Of the nine infants born with a chromosomal abnormality (Table VI), only one (triploidy, 69,XXY) had an ultrasound examination indicating severe intrauterine growth retardation.

The ages of the six women who gave birth to infants with Down's syndrome were 19, 22, 22, 25, 42, and 44 years. The last two mothers had refused genetic am-

Table VII. Congenital malformations: Miscellaneous

Anomaly	*No.*	*Abnormal ultrasound*	*Antenatal diagnosis*	*Incidence*	*Reference*
Cornelia de Lange's syndrome	1	0	0	1/>10,000	11
Nonimmune hydrops	1	1	1	1/3500	29
Sacrococcygeal teratoma	1	1	1	1/40,000	11
Placental chorioangioma	2	2	2	Microscopic, 1/100; >2 cm in diameter, 1/1000	30

Table VIII. Indications for ultrasound examination in cases with congenital malformations

Indication	*Patients (No.)*
Routine	28
Pregnancy dating	16
Rule out congenital anomaly	6
Polyhydramnios	4
Rule out placenta previa	2
Suspected growth retardation	2
Breech	1
Premature rupture of membranes	1
Fetal tachycardia	1
Large for gestational age	1
Prior stillbirth	1
Diabetes	1
Total	64

niocentesis. Infant weights at delivery were between 2680 and 3140 gm, and the gestational ages ranged from 37 to 42 weeks. One infant with Down's syndrome weighed 2160 gm and was delivered prematurely at 34 weeks' gestation to a Class B diabetic mother.

Miscellaneous congenital anomalies

Miscellaneous congenital abnormalities diagnosed antenatally included nonimmune hydrops (one case), sacrococcygeal teratoma (one case), and placental chorioangioma (two cases) (Table VII). The woman who gave birth to a 1900 gm female infant with Cornelia de Lange's syndrome at term had not had an ultrasound examination after 22 weeks' gestation. At that time, fetal size was appropriate for the stated gestational age.

Indications for ultrasound examination

Routine obstetric ultrasonography and pregnancy dating were by far the most frequent reasons for scanning in this series (Table VIII).

Although the number of fetal congenital anomalies detected in our indigenous obstetric population has remained relatively static, the number of referral patients whose fetuses have ultrasonically detected congenital anomalies has continued to increase (Table IX). Six of the 19 referral patients had "suspected congenital anomaly" as the reason for their stage II ultrasound examination (Table VIII).

Table IX. Patient base for congenital anomalies

	Year			
Patients	*October, 1979, through 1980*	*1981*	*1982*	*January and February, 1983*
Mayo Clinic	14	14	15	2
Referral	2	4	10	3

Comment

In the United States each year, 100,000 to 150,000 infants are born with a significant congenital anomaly. Of this group, 68,000 have physical malformations.[31] As demonstrated in our study, the ultrasonographer's ability to detect and diagnose specific physical abnormalities improves with experience. Indeed, it is conceivable that with technical improvements in ultrasonography we eventually will be able to diagnose most of the significant morphologic abnormalities.

Several factors are important in the ultrasonic detection of congenital malformations. First, as mentioned, there is the resolution provided by the available sonographic equipment. Although cardiac anomalies are present early in gestation, current real-time scanners do not permit an antenatal diagnosis until the last part of the second or the early part of the third trimester.[32] Neural tube defects, on the other hand, have been detected ultrasonically by 16 weeks' gestation.[33]

Second, the time in gestation when a particular abnormality manifests itself must be known. For duodenal or jejunal atresia to be diagnosed in utero, a sufficient amount of amniotic fluid must enter the gastrointestinal tract. Fetal deglutition and a stomach "bubble" have been viewed ultrasonically as early as 16 weeks.[34] The quantity of amniotic fluid swallowed at this gestational age, however, is only 2 to 7 ml per day. Hence, ultrasonic scanning in the last part of the second trimester, when the amount of gastrointestinal fluid has increased, may be necessary to exclude obstruction.[35]

Third, the ultrasonic appearance of a particular anomaly may vary among cases, depending on its severity.[36] An accurate assessment of the incidence of posterior urethral valves, for example, is difficult since demonstration of obstruction may vary from detection in utero to a pathologic diagnosis at autopsy in the adult.

Fourth, specific ultrasound criteria that permit a particular diagnosis to be made must be available. Although an irregularly shaped calvarium was detected ultrasonically in our case of sagittal craniosynostosis, the correct diagnosis was not suspected. A retrospective review of the ultrasonic examination, with the use of the cephalic index, head circumference, and abdominal circumference (techniques unavailable at the time of the scan), permitted an accurate dating of the pregnancy and consideration of the final diagnosis.

As illustrated in this review, the number of congenital abnormalities that can be detected ultrasonically is increasing almost daily. Harrison et al.[37] outlined five therapeutic alternatives once a congenital malformation has been diagnosed: (1) selective abortion, (2) correction after term delivery, (3) induced preterm delivery for early neonatal therapy, (4) prophylactic delivery by cesarean section in order to reduce fetal trauma, and (5) therapy in utero. Since the congenital anomalies that have the potential for correction in utero are relatively few and are dependent upon detection at a time when fetal therapy or surgical treatment would be efficacious, fetal intervention in utero will remain a relatively uncommon event. Hence, it is in the selection of one of the other management choices that antenatal diagnosis of a congenital anomaly will have its greatest impact.

Routine screening of all obstetric patients by real-time ultrasound for congenital malformations is currently not cost effective. However, there is a specific group of patients who should undergo a stage II ultrasound examination between 20 and 23 weeks' gestation or when the situation dictates.

1. The major anomaly rate is approximately 18% in patients with polyhydramnios. A third of the fetuses in this group will have intestinal obstruction. Other associated anomalies include anencephaly and meningomyelocele.[38]
2. Ten percent of infants delivered with intrauterine growth retardation have congenital anomalies.[39]
3. Major malformations are increased approximately fourfold in children of diabetic women.[40]
4. The incidence of fetal malformations is known to be increased in multiple pregnancies[41] and in breech presentation.[42]
5. The incidence of major anomalies has been reported to be as high as 20% in fetuses with an abnormal result on the antepartum fetal heart rate test.[43]
6. The risk of recurrence of a congenital anomaly in subsequent children is dependent upon the anomaly or syndrome involved. Routine ultrasonic screening of this subgroup would seem prudent if the anomaly in question can be detected antenatally. Unfortunately, more than 90% of malformed infants are the first affected individuals of a family.[44]

On the basis of the data outlined above, the following anatomic sites and fetal dimensions should be evaluated during each stage II ultrasound examination: (1) fetal spine and calvarium; (2) lateral ventricular: hemispheric width ratio, to exclude early hydrocephalus; (3) the fetal neck, to detect hygromas and anterior masses; (4) the chest, to look for pleural effusions; (5) the skin, to exclude edema; (6) the fetal limbs, to measure their length and evaluate movements (neurological damage may first be suspected by notation of either abnormal or unilateral absence of fetal movements)[45]; (7) four-chamber cardiac view; (8) the abdomen, to rule out ascites; (9) the stomach and intestines, for the presence of excess fluid accumulation (indicating obstruction) and the absence of abdominal masses; (10) the umbilical vein, to obtain fetal abdominal circumference (recognition of abdominal wall defects is possible at this time); (11) the fetal kidneys, scanned longitudinally and transversely, and the fetal bladder (when oligohydramnios is present, it is particularly important to visualize the bladder and hence exclude renal agenesis as a diagnostic possibility); (12) a composite assessment of gestational age–involving biparietal diameter, head circumference, abdominal circumference, estimated fetal weight, and femur length–to alert the sonographer to any discrepancies in fetal growth; (13) when there is a significant disparity in the femur length: biparietal diameter ratio,[27] additional measurement of long bones[46] to exclude the skeletal dysplasias.[47] Determination of the head: abdomen ratio and a review of intracerebral architecture may also be necessary.

In conclusion, the ultrasound examination has become an accepted part of obstetric practice. As neonatal care improves, the relative contribution of fetal malformations to perinatal mortality continues to increase. Because the cause of congenital anomalies is unknown in up to 70% of cases, preventive measures are not possible; however, early diagnosis and selection of a plan of management from the choices outlined above will reduce fetal morbidity and hence improve neonatal survival.

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Sonographic monitoring to guide the performance of postabortal uterine curettage

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This communication describes a technique of sonographically monitored uterine curettage. This method is of particular value in the management of postabortal endomyometritis associated with retained products of conception or in any situation in which anatomic variation (for instance, retroversion) makes curette insertion difficult. (AM J OBSTET GYNECOL 1985;151:51-3.)

Key words: Ultrasound, postabortal curettage

Ultrasound examination of the pelvis has been suggested as a useful tool in the management of elective termination of pregnancy in the first trimester. Sonography is useful when the gestational age is uncertain, when uterine anomalies are known to be present, or when products of conception are not obtained after attempted evacuation.[1] With real-time ultrasonography the progress of the suction cannula or curette through the uterine cervix and cavity can be visualized.

The purpose of this communication is to illustrate the use of intraoperative real-time ultrasound in the management of retained products of conception after an elective first-trimester termination of pregnancy.

Case report

A 21-year-old woman, gravida 1, para 0, underwent an elective termination of pregnancy by suction curet-

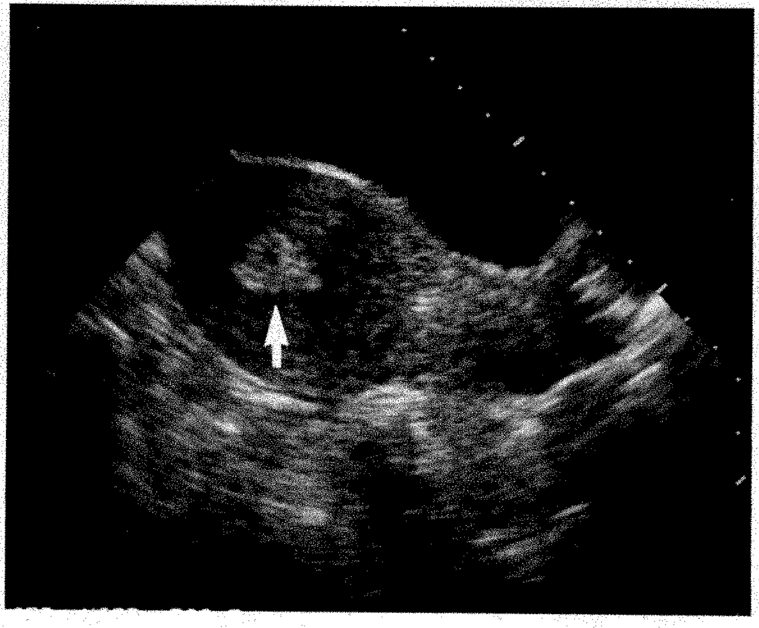

Fig. 1. Longitudinal section of the uterine fundus before operation demonstrating echogenic material *(arrow)*. The amount present is in excess of that expected from the central linear echo of the endometrial cavity alone.

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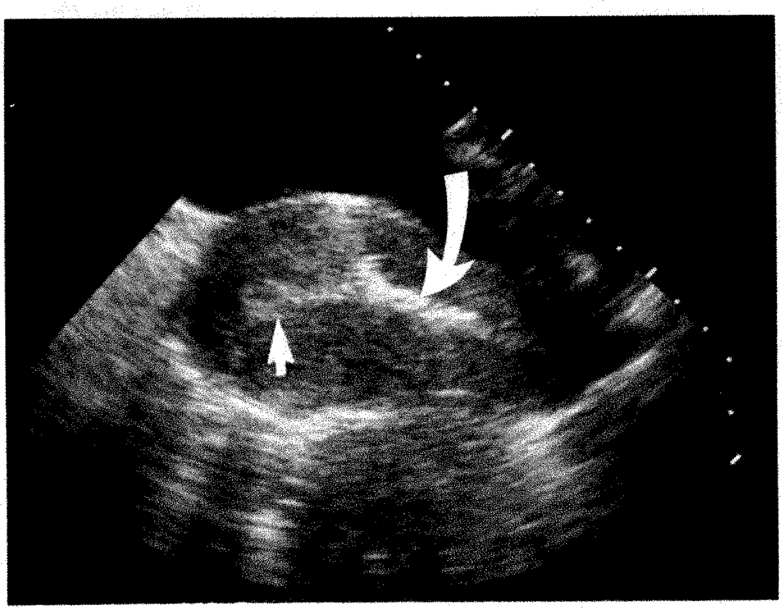

Fig. 2. Longitudinal section showing echogenic material at fundus *(straight arrow)* and curette in lower uterine segment, directed anteriorly *(curved arrow)*.

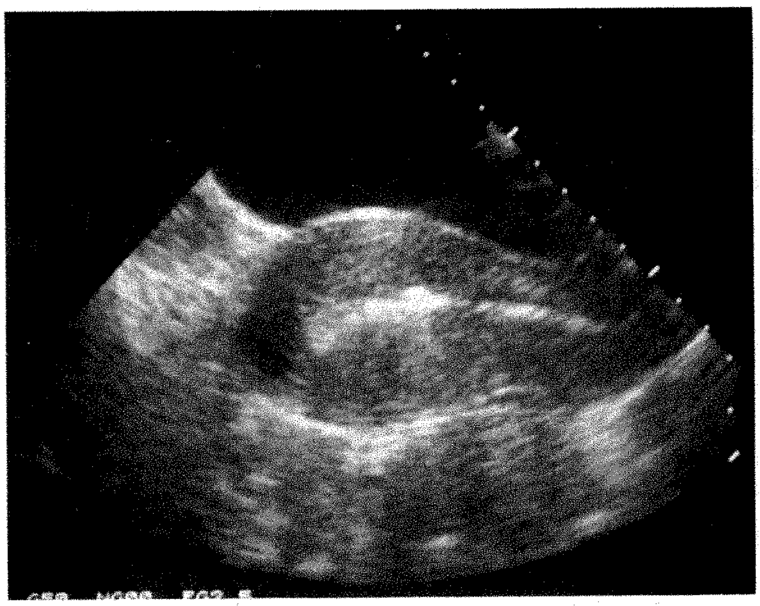

Fig. 3. Longitudinal section showing curette rotated 180 degrees and redirected into fundus.

tage at 12 weeks of gestation. The procedure was performed without difficulty. Three days later she presented to the Yale–New Haven Hospital Emergency Room complaining of fever and heavy vaginal bleeding. On examination the temperature was 103° F, with exquisite tenderness of the uterus and parametria and an open cervical os. The diagnosis of retained products of conception was entertained. The patient was unable to tolerate uterine manipulation when curettage was attempted after a paracervical block, and plans were made to take her to the operating room.

A real-time ultrasound examination of the pelvis demonstrated the presence of echogenic material in the endometrial cavity (Fig. 1), localized in the fundus. A curettage was performed; however, initially no products of conception were obtained. Real-time ultrasound demonstrated that the curette was inappropriately directed anteriorly and was not reaching the echogenic material (Fig. 2). Redirection of the curette under ultrasonic guidance resulted in the extraction of the echogenic material (Fig. 3), which proved to be necrotic products of conception on pathologic examination. Ex-

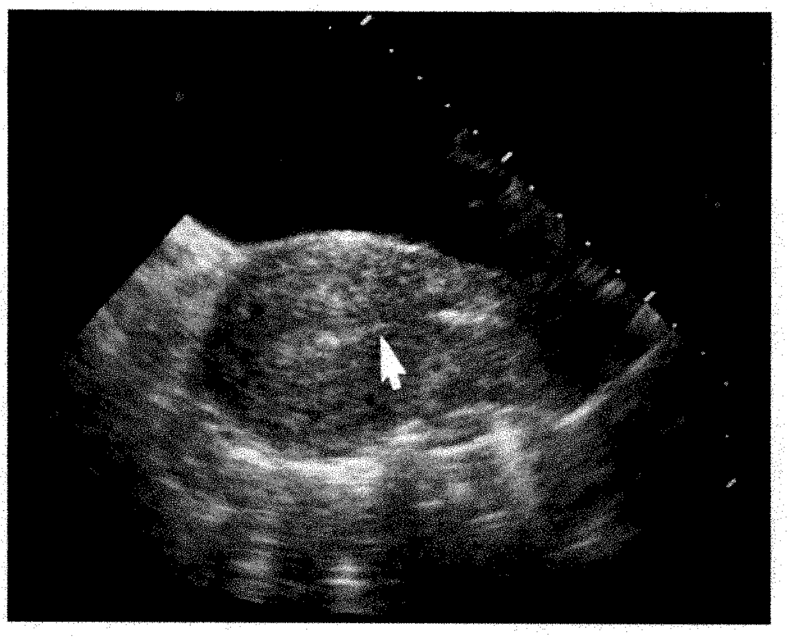

Fig. 4. Longitudinal section taken at conclusion of the procedure showing central linear echo of endometrial canal *(arrow)*.

amination of the uterus at the end of the procedure (Fig. 4) showed the endometrial cavity to be free of echogenic material.

Comment

Once postabortal endomyometritis is diagnosed the clinician is faced with the question of whether products of conception have been retained and if a uterine curettage should be performed. Generally, physical findings are used to make this judgment but the diagnostic accuracy of such clinical signs as uterine tenderness or cervical dilation in the prediction of retained products is uncertain. The accuracy of ultrasound in the diagnosis of retained products of conception has been previously reported.[2]

Our case demonstrates how ultrasound can be used to confirm the diagnosis of retained products of conception and to direct the placement of the uterine curette. This may help to assure that the uterine cavity has been appropriately emptied. In cases with concomitant endometritis or acute uterine retroversion it may help to prevent uterine perforation.

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Survey of obstetric forceps training in North America in 1981

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In April, 1983, a questionnaire was sent to all 144 United States and Canadian members of the Association of Professors of Gynecology and Obstetrics to survey residency training and current use of obstetric forceps in 1981. One hundred five programs (73%), responsible for at least 283,000 births in 1981, were subsequently analyzed. All training programs used outlet forceps and all programs but one used midforceps for delivery. Hospitals with high cesarean birth rates did not perform significantly fewer midforceps operations. Hospitals with high midforceps rates did not also have high outlet forceps rates nor did these high rates closely reflect the personal attitude to obstetric forceps of the director of the obstetric training program. Simpson's forceps were most commonly used for outlet forceps and occipitoanterior midforceps operations, whereas Kielland's forceps were selected by 76% of programs for rotational midcavity deliveries. Staff obstetricians were the primary instructors of forceps technique in the delivery room in only 50% of United States programs; all Canadian respondents reported the staff obstetrician as the principal educator in obstetric residency forceps training. (AM J OBSTET GYNECOL 1985;151:54-8.)

Key words: Obstetric forceps use, obstetric forceps training, cesarean section rates

Over the past decade, obstetric forceps delivery rates fell in the United States. In 1968, in New York, New York, 29.7% of births were forceps-assisted, whereas, in 1978, only 12.2% of babies were delivered by these operations.[1] This trend was not seen in Western European countries, where the frequency of forceps delivery steadily increased over this period.[1]

What are the reasons for this divergence of United States and European obstetric forceps practice? One explanation was the increasingly popular view, in the United States, that healthier babies would be born if fewer forceps and more cesarean deliveries were performed. Indeed, cesarean birth rates rose in America from 5.5% in 1970 to 15.2% in 1978 and reached 25% in some hospitals.[2] Cesarean section became the tenth most common operation in the United States and concern over such changes stimulated a Task Force Enquiry into Cesarean Childbirth sponsored by the National Institute of Child Health and Human Development.[1]

One basis for the decreased forceps rate, addressed in the Task Force report on cesarean childbirth, was the low priority and emphasis given to forceps delivery in some obstetric training programs.[1] Another contributing factor examined by the Task Force was the diminishing opportunity for trainee obstetricians to develop skills and knowledge in the surgical techniques of midforceps delivery.

We could find little current information in the literature about forceps training in obstetrics programs. Accordingly, the aim of this study was to determine contemporary practices and attitudes toward obstetric forceps training in North America. University teaching departments of obstetrics were surveyed for the year 1981.

Material and methods

Questionnaire. All 144 chairman members of the Association of Professors of Gynecology and Obstetrics were sent a questionnaire, together with a reply-paid envelope (Fig. 1). The questionnaire comprised nine questions concerning the obstetric training program in each hospital in 1981 and used a multiple-choice format.

One hundred eight chairmen or their obstetric training program supervisors replied, for a response rate of 75%. Three respondents currently had no obstetric residency training program and were, therefore, excluded from analysis. One hundred five replied from the United States (73%) and nine of a possible 15 from Canada. These responses constitute the data base.

Statistics. The χ^2 test without a priori hypothesis was used to examine frequencies of replies in the various groups. For 2×2 table analysis, the Yates continuous correction factor was applied.

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QUESTIONNAIRE: OBSTETRIC FORCEPS 1981

Where it applies, please mark the *SINGLE* most appropriate box to answer each question.

1. How many deliveries occurred in the obstetric resident training sections of your hospital(s) in 1981?
   <1000 ____; 1000-3000 ____; 3000-5000 ____; 5000-7000 ____; >7000 ____
2. What was the cesarean birth rate for these deliveries?
   <5% ____; 5%-10% ____; 10%-15% ____; 15%-20% ____; >20% ____
3. What percentage of deliveries in 1981 were by obstetric outlet forceps?
   <5% ____; 5%-10% ____; 10%-15% ____; 15%-20% ____; >20% ____
4. What percentage of deliveries in 1981 were by midforceps?
   0% ____; <1% ____; 1%-4% ____; 4%-8% ____; >8% ____
5. Which instruments are most commonly used by your obstetric residents for:
   a. Outlet forceps delivery? ____
   b. Occipitoanterior midforceps delivery? ____
   c. Rotational midforceps delivery? ____
6. In your program, who predominantly instructs obstetric residents in delivery room forceps technique?
   Staff obstetrician ____; Senior resident ____; No one ____
7. Does your program have an obstetric forceps mannequin course for obstetric residents?
   Yes ____ No ____
8. How important do you believe outlet forceps are to contemporary obstetric care?
   Not at all important ____; Slightly important ____; Important ____; Very important ____
9. How important do you believe midforceps are to contemporary obstetric care?
   Not at all important ____; Slightly important ____; Important ____; Very important ____
10. Do you wish a copy of the results of this questionnaire?
    Yes ____ No ____
11. Any comments or suggestions:

Fig. 1. Questionnaire to survey resident training in obstetric forceps.

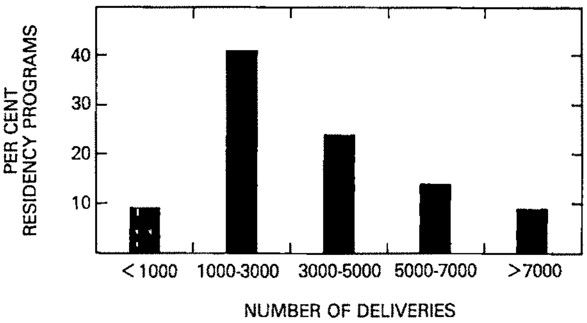

Fig. 2. Histogram of the number of deliveries in North American obstetric residency training programs.

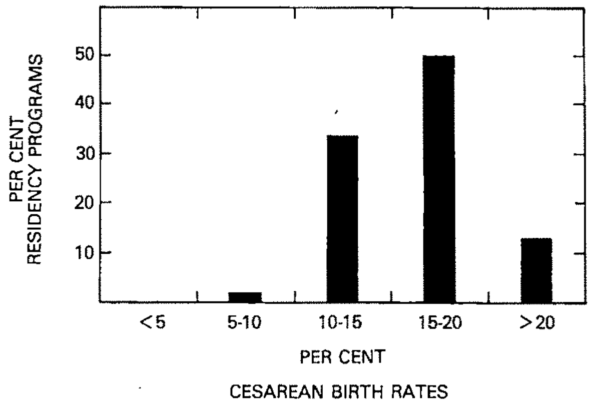

Fig. 3. Histogram of the cesarean birth rates in North American obstetric residency training programs.

Results

Figs. 2 to 5 show the number of deliveries, cesarean birth rates, outlet forceps use rates, and midforceps use rates in residency programs in North America in 1981. At least 283,000 babies were delivered in these resident teaching hospitals in that year. Fifty-three percent of residency programs performed fewer than 3000 deliveries per year, while 9% delivered less than 1000 women that year. Conversely, 24% of respondents had more than 5000 births and 9% supervised more than 7000 deliveries in 1981 (Fig. 2). Cesarean birth rates were above 15% in 65% of obstetric residency programs and in excess of 20% in 13% of respondents' hospitals (Fig. 3).

Frequency of forceps use. All hospitals reported using outlet forceps in their residency programs. The frequency of use of outlet forceps followed a Gaussian distribution (Fig. 4), with 15% of programs performing this operation in fewer than 5% or in more than 20% of deliveries.

All residency programs but one performed midforceps operations (Fig. 5). Typically (total of 82%), 1% to 4% of births were from the midcavity. Midforceps operations were undertaken for more than 4% of deliveries in 16% of programs, and three hospitals delivered more than 8% of their patients by this operation.

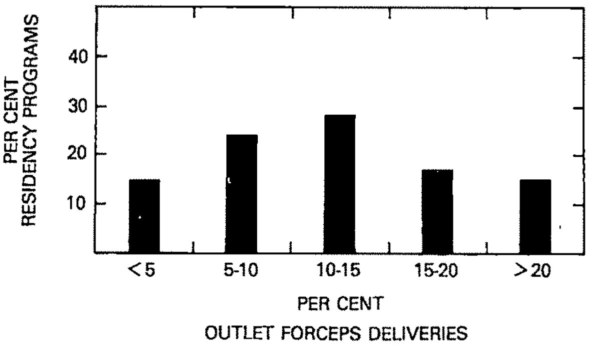

Fig. 4. Outlet forceps delivery rates in North American obstetric residency training programs.

Statistical analysis revealed that hospitals with high cesarean birth rates did not perform significantly fewer midforceps operations. Indeed, there was a trend ($p < 0.10$) for hospitals with higher cesarean birth rates to perform more, not fewer, midforceps deliveries. Programs with high midforceps use rates (>4%) did not have statistically higher outlet forceps use rates. Furthermore, busy services (>5000 births) did not perform more outlet forceps, midforceps, or cesarean operations than did less active programs.

Attitude toward forceps use. Table I displays the attitude of directors of obstetric residency programs toward forceps operations in contemporary obstetric practice. Sixty-five percent of directors believed midforceps operations were important in contemporary obstetric practice. Programs whose directors believed outlet and midforceps operations "very important" to current obstetric practice did not perform more forceps deliveries than did programs with less enthusiastic directors ($p > 0.15$).

Forceps instruments used. Table II shows the forceps instrument most commonly used for the three major regional operations. Simpson's forceps was the most-used instrument for both outlet and occipitoanterior midcavity forceps deliveries (54% and 52%, respectively). Kielland's forceps were used by 76% of institutions for rotational midcavity deliveries. Eight percent of respondents used Simpson's or Tucker-McLean forceps most frequently for midforceps rotation and delivery.

Labor and delivery instructors. In 32 programs (30%), senior residents were the primary instructors of forceps technique in the delivery room. In 18 programs (17%), senior residents and staff obstetricians were co-dominant instructors in forceps training, while in 52 replies (50%), staff obstetricians were the major teachers. Thirty-six percent of programs used a mannequin course to aid in forceps instruction for their obstetric residents.

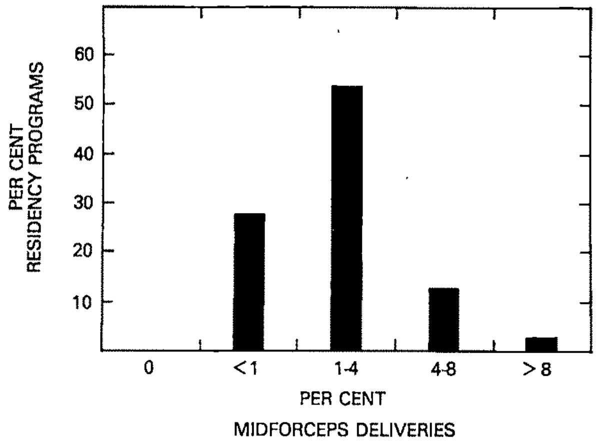

Fig. 5. Midforceps delivery rates in North American obstetric residency training programs.

Canadian and United States programs compared. The frequency of Canadian respondents (nine of 15; 60%) was lower than that of United States programs (77%). Statistical comparisons between countries could not be reliably made. All Canadian programs which replied reported that a staff obstetrician was the major instructor of residents in forceps technique. This was different from United States practice, where a staff obstetrician was the dominant instructor in only 43% of programs. Only one Canadian respondent provided a mannequin course. Canadian practice and attitudes were otherwise similar to those of the total data base.

Comment

There are obvious criticisms to the questionnaire type of inquiry: Opinions expressed are only of those who reply; the answers are difficult to validate; and there is no room for discussion, although the questions in this survey (Fig. 1) were well defined. However, there is no other satisfactory way to survey current practices and attitudes toward resident training in the use of obstetric forceps. The high response rate (75%) indicates the interest of directors of obstetric training programs in this study and their need for such information.

A major finding from this study was that midforceps deliveries were performed in all but one of the 105 obstetric training programs that responded (Fig. 5). This was surprising, given the view of several prominent American obstetricians that midforceps delivery is no longer justified in modern obstetric practice because of attendant increased perinatal morbidity and neurological disability.[3-6] Still, the use of midforceps in contemporary obstetrics remains controversial. Several recent series have reported that, with advances in conduction anesthesia, detection of fetal acidosis, and early cesarean section for true cephalopelvic disproportion,

Table I. Obstetric program directors' attitudes toward use of forceps in contemporary obstetrics (results are percentages)

Type of forceps	*Not important*	*Slightly important*	*Important*	*Very important*
Outlet	2	8	54	36
Midforceps	5	30	47	18

Table II. Type of forceps most commonly used in obstetric teaching programs (results are percentages)

	Type of operation		
*Forceps**	*Outlet*	*Occipitoanterior midcavity*	*Rotational midcavity*
Simpson	54	52	8
Tucker-McLean	20	21	8
Luikart	11	10	1
DeLee	4	4	0
Elliott	4	2	0
Divergent	2	1	2
Kielland	0	7	76
Barton	0	0	2
Multiple†	44	33	27

*The remaining respondents either gave no response or voluntarily reported using vacuum extraction most often for outlet operations (one reply) and for occipitoanterior midcavity operations and rotational midcavity deliveries (10 replies).

†Respondent indicated two or more types of forceps per category were used.

midforceps results are equivalent to the outcome of cesarean section.[7, 8] Moreover, Chiswick[9] followed up a group of 101 babies born in 1979 by Kielland's forceps delivery and found no perinatal morbidity and no handicap in any baby at 2 years of age.

Several clinical tenets or questions about midforceps operations can be addressed by this study. Do hospitals with high cesarean birth rates perform fewer midforceps deliveries? We found no statistical evidence of this (Figs. 3 and 5). Although cesarean section is a fashionable alternative to performance of a midforceps vaginal operation, the decline in frequency of midforceps procedures does not seem to be a primary reason for the recent large increase in the cesarean birth rate.[1, 2]

Are hospitals with high midforceps use rates "forceps oriented" or "forceps dominated" and, if so, is this because of a personal belief in forceps use on the part of the director of the obstetric training program? The answer appears to be no. Programs in which midforceps operations were performed frequently (>4%) did not also have significantly more outlet forceps operations. The attitude of the supervisor of the obstetric residency program toward the importance of forceps operations in modern obstetrics had no correlation with his program's rates of forceps deliveries (Table I).

The mode of selection of the type of forceps most commonly used for midcavity and outlet forceps operations in obstetric training was of interest (Table II). There was no indication that design and function were relevant to the selection of the instrument used. It appeared, from the responses, that the choice of the primary forceps used in most programs was by habit and past experience of the instructing personnel.

The major difference in Canadian and United States obstetric training programs was that in all Canadian schools a staff obstetrician was the principal instructor of forceps technique in the delivery room. Only 50% of United States programs used staff obstetricians as the primary instructor; chief residents were the major teachers of obstetric forceps technique in up to 50% of United States residency programs. This finding is of concern, in view of the chief residents' short experience in selecting cases appropriate for midforceps delivery. These results are in agreement with the view of the Task Force on Cesarean Childbirth concerning the low priority and emphasis given to forceps delivery in some obstetric training programs.[1]

Replies to this survey establish that outlet and midforceps operations remain an integral part of North American obstetric residency training. Furthermore, outlet forceps and midforceps were still considered important or very important in modern obstetric practice by 90% and 65% of obstetric residency program directors, respectively. It is ironic that two major postgraduate obstetric texts devote fewer than 2% of their pages to forceps delivery.[10, 11]

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Initial instruction in the pelvic examination in the United States and Canada, 1983

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Initial instruction in the pelvic examination in the United States and Canada was reviewed by questionnaire. Seventy-two percent of the 116 responding medical schools taught this material in the second year, two-thirds with a specific course devoted to the subject. Ninety-three percent used "live models" or "teaching associates" in place of, or in addition to, traditional methods such as the examination of clinic patients. The use of teaching associates was uniformly rated as an extremely effective educational method. A continued trend toward use of an educational methodology that emphasizes communication as well as psychomotor skill instruction is identified. (AM J OBSTET GYNECOL 1985;151:58-60.)

Key words: Teaching associate, gynecologic teaching associate, pelvic examination, medical examination

"Pelvic examination" has become a phrase for several aspects of women's health evaluation, including the obstetric-gynecologic and sexual histories as well as the breast and pelvic examinations. Teaching the "pelvic examination" to medical students has thus been difficult, because both psychomotor and communication skills must be mastered.[1, 2] Following Barrow's work with stimulated patients,[3] many medical educators turned to variations on this method to teach the complex psychomotor and communication skills involved.[4-6] They found that the use of "live models," women trained to allow multiple examinations, greatly improved learning in both areas. When these women received special training in both the physical examination and communication skills, they became the teachers, providing students with immediate feedback about both communication and psychomotor skills performance. Student learning was further facilitated. An additional benefit was that these women could be selected for normal anatomy as well as ease of examination, giving students the opportunity to "learn the normal" before being asked to examine and interpret findings on actual patients who may be relatively uncooperative or have pathologic findings.[1, 2, 7-9]

The present study was designed in an attempt to determine how the pelvic examination is currently taught to medical students in the United States and Canada. A questionnaire was sent to all medical schools seeking this information. This report describes the results of that survey.

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Table I. Year of first instruction in pelvic examination (n = 116)

Year	*No. of schools*	*%*
1	16	14
2	83	72
3	15	13
4	2	2

Material and methods

A nine-item questionnaire was designed with three categories of questions: in what year and by whom instruction was given; what emphasis was given to this educational component by the school; and how the faculties rated the effectiveness of the materials and methods they used. The questionnaire was sent to the deans in charge of curriculum of 143 medical schools in the United States and Canada with the request that it be forwarded to the course director for the program in which the initial instruction in the pelvic examination was given.

The first category of questions included items regarding the place in the curriculum of the first instruction, class size, hours alloted to the instructional component, and the departmental affiliations of the course directors and instructors. In the second category we asked whether there was a specific component of the curriculum dealing with student and patient feelings and attitudes about the pelvic examination (which if present is taken as evidence of a specific emphasis on communication skills) and whether there was a specific budget for the initial instruction in the pelvic examination. The last category was addressed through a rating scale listing twelve instructional materials and methods: lectures, discussion sessions, audiovisual materials, texts, and the examination of artificial pelvic models such as "Ginny", prostitutes, anesthesized patients, clinic patients, hospitalized patients, live paid pelvic models (who do not provide feedback to the student during an examination on themselves), and live paid teaching associates (who provide immediate feedback and instruction to the student during an examination on themselves). The rating scale constituted of five values (ineffective, somewhat effective, effective, quite effective, and extremely effective) and a category for "not applicable." Respondents were instructed that evaluation should be made only of those materials or methods actually used. A final item asked about the basis on which the assessments were made (student feedback, test results, clinical performance, and faculty feedback).

Results

Eighty-one percent (116) of the 143 schools receiving questionnaires responded. In 82 schools (72%) initial instruction in the pelvic examination occurred in the second year, the traditional time for the introductory physical diagnosis course, with the majority of the remaining schools offering their courses in the first year or third year (Table I). Seventy-nine schools offered 2 to 4 hours of instruction to a class averaging 120 students per year, the largest class being 250 students and the smallest 25 students (Table II). Seventy-eight (58%) of the 135 course directors identified were obstetrician-gynecologists, with the majority of the remaining 57 course directors being internists. Of the 145 instructors identified, 94 were obstetrician-gynecologists, with family practitioners and internists making up the largest other groups (Table III).

Table II. Hours devoted to initial instruction in pelvic examination (n = 112)

Total No. of hours	*No. of schools*	*%*
1	10	9
2	24	21
3	33	29
4	22	20
5	4	4
6	8	7
7-15	8	7
≥16	3	3

Table III. Faculty affiliations of directors and instructors of courses providing initial instruction in pelvic examination

	Course director		*Instructor*	
Department	*No.*	*%*	*No.*	*%*
Obstetrics and gynecology	78	58	94	65
Medicine	41	30	18	12
Surgery	1	0.7	2	1
Family practice	8	6	16	11
Pediatrics	0	0	3	2
Psychiatry	1	0.7	2	1
Other	6	4	10	7

One hundred twelve schools responded to the question about a specific curriculum component of the initial pelvic examination course dealing with student and patient feelings and attitudes, with 72 (64%) indicating that they had such a component. Sixty-two of the 114 responding schools (54%) indicated they also had a specific budget for the initial instruction in the pelvic examination.

Eighty-one percent of schools use lectures and 84% audiovisual aids as part of their programs, generally rating them as "effective." Sixty-eight percent of schools used discussion sessions and 63% books, with the former tending to be rated as more effective than the latter. One fourth to one third of schools used anes-

Table IV. Materials and methods used in initial instruction in the pelvic examination (n = 116)

	Users		*Rater scale of effectiveness of material or method used*									
			Ineffective		*Somewhat effective*		*Effective*		*Quite effective*		*Extremely effective*	
Items	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Lecturers	94	81	4	4	25	27	36	38	21	22	8	9
Discussions	79	68	4	5	9	11	26	33	20	25	20	25
Audiovisual	98	84	2	2	10	10	37	38	34	35	15	15
Books	73	63	6	8	23	32	29	40	10	14	5	7
Pelvic models ("Ginny")	60	52	8	13	16	27	15	25	16	27	5	8
Prostitutes	3	3	2	66	0	0	0	0	1	33	0	0
Anesthetized patients	27	23	2	7	0	0	7	26	7	26	11	41
Clinic patients	40	34	1	0	5	13	5	13	18	46	11	28
Hospitalized patients	32	28	1	3	2	6	5	16	16	50	8	25
Live models	18	16	1	6	1	6	3	17	7	39	6	33
Teaching associates	90	78	3	3	0	0	0	0	8	9	74	82

thetized, clinic, or hospitalized patients, with three schools still using prostitutes for examination. Users of these methods tended to rate them as at least effective (Table IV).

Fifty-two percent of schools use artificial pelvic models (e.g., "Ginny") with a uniform distribution on the rater scale about the "effective" rating. One hundred eight (93%) of schools used either live models (18 schools, or 16%) or teaching associates (90 schools, or 77%). Both methods received high ratings, with the teaching associate method receiving the highest rater evaluation (82% choosing "extremely effective"). Other methods mentioned included female students examining each other with no provision for male students in one school and students being given the optional opportunity to examine each other in another school, regardless of sex. In the latter school, about 25% of students elected to participate each year. The judgments made in the rating were predominantly based on student feedback (95%), with test results, clinical performance, and faculty feedback used by 16%, 43%, and 56% of respondents, respectively.

Comment

Initial instruction in the pelvic examination continues to be a part of the second year curriculum in most medical schools, a time traditionally devoted to initial instruction in physical diagnosis. Faculty participation by specialty predominantly involved obstetrics and gynecology and internal medicine. The former is presumed to reflect the association of obstetrics and gynecology with the pelvic examination and the latter the traditional involvement of internists in basic physical diagnosis courses.

There is a continued trend towards the use of specially trained women instructors for the initial instruction in the pelvic examination. Effectiveness evaluation indicates a high degree of satisfaction with these "gynecologic teaching associates" as instructors of both communication and psychomotor skills. The number of schools with specific budgets for these courses demonstrates the importance placed on these aspects of medical education in women's health.

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Antepartum fetal heart rate testing

XII. The effect of manual manipulation of the fetus on the nonstress test

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A prospective study of 790 patients was performed in order to examine the role of manual manipulation of the fetus in the nonstress test. The patients were assigned randomly to two groups based on the last two digits of their hospital number. The odd-numbered patients underwent manual manipulation of the fetus prior to the onset of the nonstress test; the even-numbered patients did not. There was no significant difference between the two groups with respect to the indications for testing and the total number of tests. There was no statistically significant difference between the two groups with respect to the ratio of reactive to nonreactive nonstress tests and the mean duration of testing. Simple manual manipulation of the fetus does not seem to change the outcome in antepartum fetal heart rate testing when the nonstress test is the primary one. (AM J OBSTET GYNECOL 1985;151:61-4.)

Key words: Fetal manual manipulation, nonstress test

The use of antepartum fetal heart rate testing in the form of the nonstress test (NST) and contraction stress test (CST), singly or in combination, has become an integral part of modern obstetric practice.

The role of various methods of stimulation to change fetal state has been studied extensively. Sound, glucose, and simple manual palpation have been used in an attempt to change the "rest-activity" state of the fetus. This is done in an attempt to distinguish the truly compromised fetus that is inactive because of possible central nervous system hypoxemia from the fetus that is undergoing a physiologic rest period. Conflicting reports regarding the efficacy and clinical application of these various techniques abound in the literature.[1-9] This study examines the role of simple manual manipulation of the fetus as a stimulus to increase fetal activity.

Material and methods

During the period of July 1, 1978, through June 30, 1979, 1810 NSTs were performed on 790 patients. The indications for testing are listed in Table I. The protocol for testing has been described previously.[7] Briefly, a reactive NST was defined as two accelerations of the fetal heart rate (FHR) ≥15 bpm above the baseline FHR, lasting for 15 seconds, associated with fetal movement, in a 20-minute period. A nonreactive NST failed to meet these criteria in two consecutive 20-minute periods. If a test was nonreactive after 20 minutes, manual manipulation of the fetus was performed for 60 seconds. This was done by grasping both poles of the fetus and moving the fetus back and forth in a rocking motion. The following 4 minutes of FHR tracing were disregarded and a further 20-minute segment of FHR tracing was recorded. After 40 minutes, the test was classified as reactive or nonreactive. If the test was nonreactive, it was repeated on the same day or a CST was performed. Reactivity occurring during a CST was interpreted as a "reactive NST with Pitocin," and the oxytocin infusion was discontinued.

Patients with hospital numbers ending with an odd number underwent manual manipulation of the fetus for 60 seconds prior to the beginning of the first 20-minute NST segment. Patients in this group with a reactive NST at the end of 20 minutes were designated as group A/stim. 1. Patients with nonreactive tests after 20 minutes underwent manual manipulation of the fetus as previously described. This manipulation was designated as stim. 2, and these patients were referred to as group A/stim. 1 and 2 (Fig. 1).

Patients with hospital numbers ending with an even number did not undergo manual manipulation of the fetus prior to the initial 20-minute segment. Those pa-

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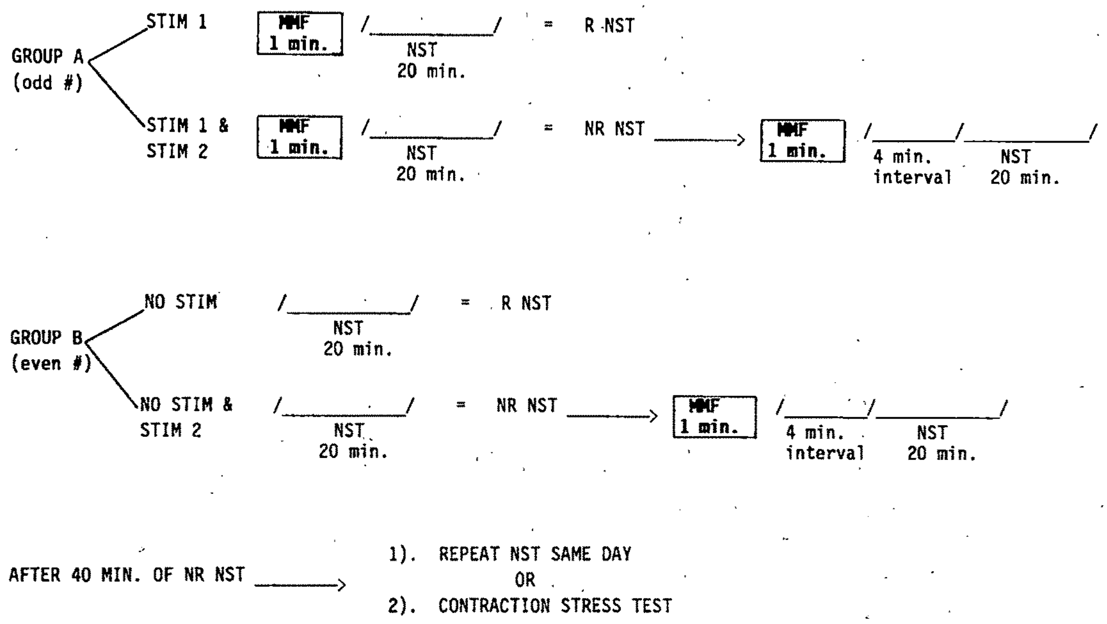

Fig. 1. Definition of test groups. *MMF* = Manual manipulation of fetus.

Table I. Indications for testing

	*Group A**		*Group B**		*Total*	
Diagnosis	*n*	%	*n*	%	*n*	%
Suspected postdates	382/922	41	356/888	40	738/1810	41
Diabetes mellitus						
Class A	38/922	4	36/888	4	74/1810	4
Class B	147/922	16 } 25%	144/888	16 } 24%	291/1810	16 } 24%
Classes C through R	46/922	5	35/888	4	81/1810	4
Suspected intrauterine growth retardation	122/922	13	117/888	13	239/1810	13
Hypertensive disorders	116/922	13	70/888	8	186/1810	11
Miscellaneous	71/922	8	130/888	15	201/1810	11
Total	922/1810	51	888/1810	49		

*p = NS (χ^2).

tients with reactive tests at the end of the first 20 minutes were designated as group B/no stim. Patients with nonreactive tests after 20 minutes underwent manual manipulation of the fetus and were designated as group B/no stim. and stim. 2 (Fig. 1).

A reactive NST was classified as normal and a nonreactive NST as abnormal. Further subgroups were created depending on the outcome of the CST in the nonreactive NST cases. Following initial classification of the tests by the authors who are nursing personnel, P. B., D. McC., and M. S., the tests were interpreted by two of the authors, M. L. D., and J. G.

Indications for testing were essentially similar in group A and group B (Table I). The majority of tests were done because of suspected postdates pregnancy (40% to 41%), followed by diabetes (24% to 25%) and suspected intrauterine growth retardation (13%).

Testing was performed at gestational ages ranging from <30 weeks to >42 weeks. The number of tests performed at each gestational age is listed in Table II.

Results

Of the 1810 NSTs performed, 1633 (90%) were reactive and 177 (10%) were nonreactive. Results were analyzed in terms of groups A and B, and special attention was given to group A/stim. 1 and 2 and group B/no stim. and stim. 2.

In group A, 400 patients (51% of all patients tested) underwent 922 NSTs; 822 tests (89%) were classified as normal and 100 tests (11%) as abnormal. In group B, 390 patients (49% of all patients tested) underwent 888 NSTs; 811 tests (91%) were reactive and 77 (9%) were nonreactive. Thus there was no statistically significant difference in the reactive NST:nonreactive NST ratio between the two groups.

In group A/stim. 1, the mean duration of testing was

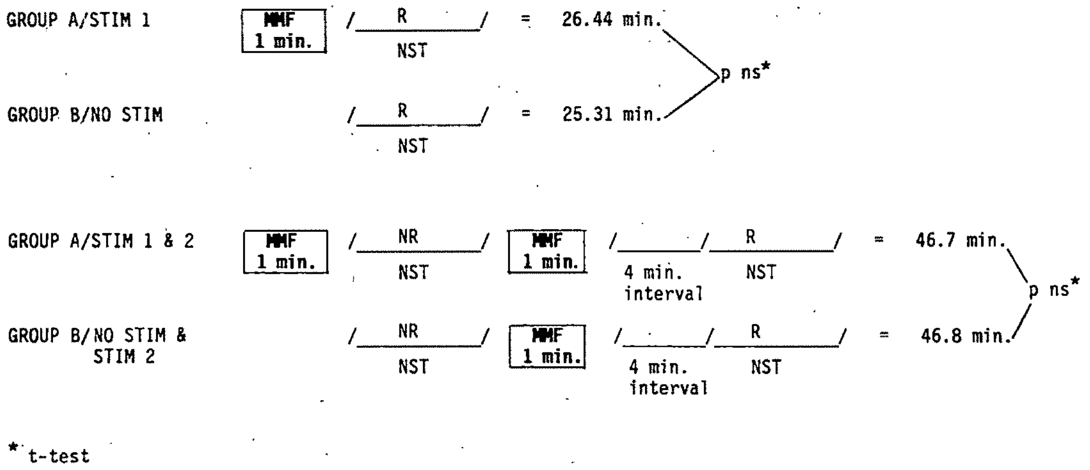

Fig. 2. Mean duration of testing. *MMF* = Manual manipulation of fetus.

Table II. Test results by gestational age and test group

Gestational age (wk)	Group A					Group B				
	No. of tests	Reactive NST		Nonreactive NST		No. of tests	Reactive NST		Nonreactive NST	
		n	%	*n*	%		*n*	%	*n*	%
<30	6	3	50	3	50	5	1	20	4	80
30-32	26	23	88	3	12	20	19	95	1	5
32-34	95	88	93	7	7	82	73	89	9	11
34-36	124	114	92	10	8	135	129	96	6	4
36-38	132	122	92	10	8	136	127	93	9	7
38-40	115	101	88	14	12	124	114	92	10	8
40-42	208	189	91	19	9	179	165	92	14	8
>42	213	180	85	33	15	201	179	89	22	11
Total*	919†	820	89	99	11	882‡	807	91	75	9

*p = NS (χ^2).
†Three NSTs of unknown gestational age omitted: two reactive NSTs, one nonreactive NST.
‡Six NSTs of unknown gestational age omitted: four reactive NSTs, two nonreactive NSTs.

26.44 minutes; 499 of 922 NSTs (54%) were reactive in the first 20-minute period of testing. In group A/stim. 1 and 2, the mean duration of testing was 46.7 minutes; 326 of 922 tests (35%) required manual manipulation of the fetus after 20 minutes of nonreactive tracing and subsequently became reactive. The remaining 97 NSTs (2%) had sufficient spontaneous uterine contractions to qualify for a CST and thus did not require stim. 2.

In group B/no stim., the mean duration of testing was 25.31 minutes; 526 of 888 NSTs (59%) were reactive in the first 20 minutes of testing. The mean time for group B/no stim. and stim. 2 was 46.8 minutes; 347 of 888 NSTs (39%) required manual manipulation of the fetus after 20 minutes of nonreactive tracing. The remaining 15 NSTs (2%) had a spontaneous CST and did not require stim. 2.

There was no statistically significant difference in the mean time for a test between group A/stim. 1 and 2 and group B/no stim. and stim. 2 (46.7 versus 46.8 minutes). In addition, the difference in the mean duration of testing between group A/stim. 1 and group B/no stim. was not statistically significant (26.44 versus 25.31 minutes) (Fig. 2).

Outcome

There were two fetal deaths within 1 week of a reactive NST. Both deaths occurred in the suspected postdates group. In the first case, a reactive NST was obtained on September 11, 1979. A serum unconjugated estriol analysis done on the same day was reported as 6.8 ng (normal, ≥12 ng/ml). A repeat estriol analysis on September 14, 1979, was 5 ng. Protocol required that antepartum fetal heart rate testing be performed on that day. However, the patient could not be reached for the following 3 days, and when she came in on September 17, 1979, the fetus had died. At delivery the stillborn infant weighed 2850 gm and was grossly normal with no nuchal cord. Cord length was 36 cm. The placenta weighed 340 gm and had multiple areas of fibrosis.

In the second case of antepartum fetal death, the patient had a nonreactive NST followed by a negative CST on October 30, 1979. The serum unconjugated estriol level was 9.4 ng. On November 3, 1979, repeat analysis of serum estriol was 7.9 ng, and a reactive NST was obtained. On November 5, 1979, the patient noted absence of fetal movement, and on November 11, 1979, she returned with a documented fetal death. A grossly normal stillborn infant was delivered, with a weight of 3340 gm, meconium staining, and a loose nuchal cord. The placenta weighed 570 gm and had focal calcifications; a small placental separation with organized clot that approximated 5% of the placental surface was noted. Cord length was 56 cm.

Comment

Various methods of stimulation of the fetus have been proposed to arouse the fetus from a so-called sleep cycle or rest-activity cycle. A change of maternal position or physical activity has not been reported to change rates of fetal motion.[1]

A number of studies have reported an increase in fetal activity related to increased levels of maternal glucose following oral glucose ingestion.[2-4] However, recent data have shown that fetal body motion is not increased following a recent meal[5, 6] or a standard oral glucose load.[7] In addition, no relationship between maternal blood glucose levels and the incidence of reactive NSTs could be demonstrated.[8, 9] The increase in respiratory movement following a maternal glucose challenge may explain the seemingly contradictory data.[10]

An increase in fetal activity with associated increase in heart rate has been demonstrated following sound stimulation[11, 12] and light stimulation.[13, 14] These stimuli have not been used on a large scale, and their clinical applicability awaits further investigation.

These maneuvers have all been used to decrease the time spent obtaining a normal NST. An added benefit supposedly has been a lowering of the high false abnormal rate in antepartum fetal heart rate testing. Our data would suggest that simple manual manipulation of the fetus neither decreased the time required for performance of a NST nor changed the reactive NST:nonreactive NST ratio.

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Triploidy and chromosomes

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In a total sample of 105 triploid spontaneous abortions and live-born and stillborn infants, the parental origin could be determined in 77%. Dispermy was the most common cause of this abnormality. Among the digynic triploids 69% resulted from retention of the second polar body. Parental ages did not differ from those of the general population except for 10 aneuploid triploids with significantly elevated parental ages. In five sibships, simple aneuploidy in another pregnancy was observed, four of them being potentially viable. Two sisters had triploid conceptions. There were four twin pregnancies, a frequency of one in 26. Only two triploids had an XYY sex chromosome complement, one of which was mosaic with loss of an autosome and the other was a chimera. A frequency of almost 50% of mothers exposed to preconception abdominal radiation is suggestive of an association between radiation and triploidy and requires further investigation. (AM J OBSTET GYNECOL 1985;151:65-9.)

Key words: triploid, chromosomes, spontaneous abortion, twins

A relatively common form of chromosome abnormality among humans is triploidy, the presence of an extra complement of chromosomes. Most triploids abort spontaneously in early pregnancy, and those that proceed to a live birth do not survive the neonatal period. The results of a systematic cytogenetic survey of triploids are described here.

Material and methods

Most of this sample of 105 triploids were found among a consecutive series of early spontaneous abortions collected over a 6½-year period from 1977 to 1984 by the obstetric staffs of three Hamilton hospitals. The remainder of the triploids are abortuses sent from regional hospitals and stillborn and live-born infants referred because of suspected chromosome abnormalities.

In the Hamilton hospitals aborted specimens are routinely collected into clean, dry air-tight containers and delivered with heparinized maternal blood samples to the cytogenetics laboratory. Weekend specimens are refrigerated until delivery. After tissue from the fetus and/or extraembryonic membranes is taken for culturing, the remaining specimen is returned to the hospital of origin for pathologic assessment. Out-of-town specimens are treated in similar fashion and transmitted as quickly as possible by bus. If desiccation might be a problem, the specimen is covered with water-dampened gauze. Peripheral blood samples are obtained from live-born infants.

Aborted tissues are first exposed to antibiotics for 30 minutes, then rinsed, minced, and pipetted into several tissue culture dishes, large and small, and fed with fresh medium (α–minimal essential medium with 12% fetal calf serum). The cultures are grown in a carbon dioxide incubator and harvested after a healthy growth of cells is evident, usually 2 to 4 weeks later. Fetal and placental tissues are cultured separately because of possible maternal contamination of the latter.

When a chromosome abnormality is found, the parents are referred by their physicians for karyotyping. The nature of the chromosome defect is discussed with the parents, and genetic counseling is provided. Anamnestic data are not made available to the technologists in order to avoid any bias in the interpretation of chromosome variants.

Slides of chromosome preparations are stained with Atebrin for precise identification of any abnormalities, classification of inherited variants, and detection of more than one cell line. The variants are classified independently by at least two and usually three technologists and decisions made regarding the parental contributions to the triploidy. If any doubts are expressed, the family is classified as noninformative.

Results

Total sample of triploids. During this period of the abortion survey, a total of 1201 specimens were suc-

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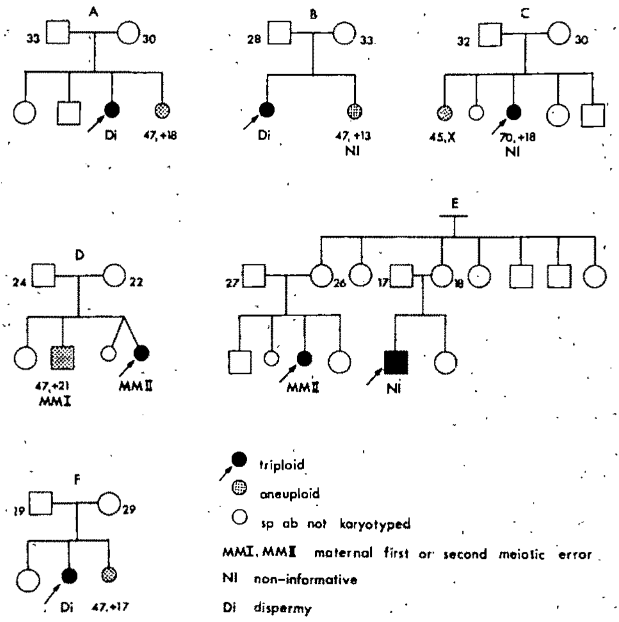

Fig. 1. Diagrams of six families with recurrence of chromosome abnormalities. All probands are triploids. Parental ages given are those at birth of each proband.

cessfully cultured, of which 596 (50%) had abnormal karyotypes. Among the abnormal specimens were 93 (16%) triploids. In addition, five abortions from the regional hospitals and one stillborn and six live-born infants brought the total number of triploids to 105 (Table I). Included among the abortions were 10 aneuploid triploids: seven were hypertriploid and three were hypotriploid. Blood specimens were obtained from 104 mothers and 92 fathers. Seven noncooperating familes, five single mothers, and one case each of artificial insemination and questionable paternity accounted for 14 incomplete families. The XXY:XXX:XYY sex ratio was 57:44:2. Two others were 68,XX.

Parental source of the meiotic error. There were 81 informative families. Dispermy accounted for 33 (63%) of the diandric triploids; variants for the remaining 19 were consistent with fertilization by diploid sperm (Table II). However, dispermy could not be ruled out with certainty and is probably the main source of the error even in these families. When paternal blood samples are not available, only exclusion of maternal origin is possible. Thus, omitting five such families, the ratio of paternal to maternal origin of the extra chromosome complement was 47:29, or 62% diandry, under the assumption that crossing over has not occurred between the centromere and the informative fluorescent variant.

Among the diandric triploids there were 19 specimens that were described as partial moles from gross examination. One other partial mole was the only one

Table I. Description of 105 families with triploid progeny

	Sex chromosome complement			
Q-band variants	*XXY*	*XXX*	*XYY*	*XX*
Informative families	44	33	2	2
Noninformative families	4	4		
Poor fetal Q-bands	3	5		
Maternal blood only*	6 [2]	1 [3]		
No parental blood	—	1		
Total	57	44	2	2

*Five cases (in brackets) are included among informative families.

with two maternal sets of chromosomes. In addition to the triploids, there were two diploid classical moles. As in other reported series,[2, 3, 4] only paternal chromosomes were present. All variants were homozygous, indicating that one haploid set of paternal chromosomes had doubled and all maternal chromosomes were lost. These results are consistent with the suggestion that almost all moles, whether partial or complete, are diandric in origin.[5]

The digynic triploids were mainly due to retention of the second polar body. Our frequency of 69% second meiotic error does not agree with the suggestion that retention of chromosomes during first division is the usual mechanism contributing to triploidy.[6] Failure of chromosomes to separate during second meiotic division was clearly demonstrated by Brennan and Carr[7] in all three of their maternally derived triploids. Since their sample was obtained at an earlier date from the same hospitals as was our series (their Case b is included here), it is possible that the high frequency of second meiotic error may be characteristic of this geographic region. However, it would appear more likely that the differences are due to the small sample sizes of informative triploids as yet available.

Parental ages. The mean ages for mothers (26.4 years) and fathers (28.9 years) of the triploids do not differ from the mean ages for the Ontario population[8] (Table III). As with other series[9, 10, 11] the mean maternal age was similar to that of karyotypically normal abortuses. The maternal age of digynic triploids showed a slight but insignificant increase over those of diandric origin.

There was a highly significant increase in parental ages of 10 triploids with superimposed aneuploidy ($p < 0.0001$). Seven were hypertriploid and three hypotriploid (Table IV). Similar increases in maternal ages have been reported in five cases[12, 13] but not in four others.[14] The greatly elevated parental ages must be related to the aneuploidy. Unfortunately we could not determine the parental origin of the extra chromosome in the hypertriploids. Two cases of 68,XX arose

Table II. Parental origin of the extra haploid chromosome complement in informative triploid families

	Maternal origin		*Paternal origin*		
Chromosome complement	*First meiotic division*	*Second meiotic division*	*Dispermy*	*First meiotic division or dispermy*	*Second meiotic division or dispermy*
69,XXY*	6	4	17	10	0
69,XXX*	3	11	10	0	6
69,XYY*(SA)	0	0	2	0	0
Live-born or stillborn	0	1	1	3†	0
Aneuploid triploids	0	4†	3	0	0
Total	9	20	33	13	6

*Spontaneous abortion.

†Includes cases reported previously.[1,7]

Table III. Mean parental ages of the triploids

	Total samples (n)	*Mean maternal age (yr)*	*Mean paternal age (yr)*
Total	105	26.4 ± 0.4	28.9 ± 0.5
Live-born and stillborn	7	25.0 ± 1.4	27.1 ± 2.0
Aneuploid triploids	10	32.1 ± 0.9	35.3 ± 1.5
Total without aneuploidy	95	25.7 ± 0.4	28.1 ± 0.6
Informative digyny	29	28.1 ± 0.4	30.1 ± 1.4
Informative diandry	52	26.0 ± 0.8	29.1 ± 0.6
Spontaneous abortions with normal karyotypes	575	26.3 ± 0.2	
Ontario population, 1980[8]		26.2	29.3

from retention of the second polar body. Since the ovum and second polar body have the same complement of chromosomes, loss of one sex chromosome probably occurred in the sperm.

Golbus[15] suggested that decreased ability to select against abnormal conceptuses in mice may be a function of increasing maternal age. The same possibility was proposed for humans[16] to account for the greater increases in maternal age of live-born trisomies compared with spontaneous abortions even when the trisomies are paternally derived. Hook,[17] however, found little evidence of relaxed selection with age. Triploids appear to provide a good test system because of the wide range of timing of fetal loss. Maternal ages did not differ between fetuses ≤12 weeks of age (25.6 years) and >12 weeks, which were mainly missed abortions (26.2 years). Mothers of affected infants were the youngest in our series of triploids (Table III).

Twins. Twinning was confirmed by karyotyping in two cases consisting of only fragmented tissue. Comparisons of Q-band variants ruled out maternal contamination and mosaicism. Both were dizygotic, one in each set being triploid and the other a normal female. Two other triploid abortions were reported clinically as twin pregnancies even though the specimens received were singletons. One twin pair was diagnosed by ultrasonography. Of the other pair, the first was lost at home before admission to hospital. The frequency of twinning in this series (1:26) agrees with the frequency estimated in early spontaneous abortions.[18]

Two other triploids had two cell lines. One was mosaic 68,XYY,-15/69,XYY with a frequency ratio of 80:20 respectively. The other with 46,XX and 69,XYY clones could be explained only as a chimera when twinning, mosaicism, and maternal contamination were all ruled out.

Pregnancy histories. All traceable families were contacted for follow-up studies. With the exclusion of the triploid progeny, there were 193 conceptions among which 45 (23%) were spontaneous abortions. Among 108 pretriploid conceptions, 30 (28%) were spontaneous abortions and 12 were therapeutic abortions. Eight mothers opted for genetic amniocentesis of subsequent pregnancies, all normal, and seven mothers requested tubal ligation.

Five mothers had another pregnancy with chromosome aneuploidy (Fig. 1). In the sibship with Down's syndrome (Fig. 1, *D*), both abnormal conceptuses resulted from malsegregation of maternal chromosomes. Two triploids were the progeny of sisters (Fig. 1, *E*). In these six families there were two abnormal live births, and all abnormal abortions, except the one with trisomy 17, were potentially viable. In a series with two consecutive abortions karyotyped by Boué et al.,[19] there

Table IV. Triploids with superimposed aneuploidy

Karyotype	*Maternal age (yr)*	*Paternal age (yr)*	*Fetal age (wk)*	*Type of specimen*	*Parental origin*
70,XXY,+8	31	33	12	Partial mole	Paternal dispermy
70,XXY,+21	37	43	9	Intact sac with fetus	Maternal second meiotic division; tetrasomy 21 noninformative
70,XXY,−13,+3,+14	34	32	?	Intact empty sac	Father not available; studies incomplete
70,XXX,+2	36	44	12	Fragmented tissue	Maternal second meiotic division
70,XXX,+2	28	30	22	Partial mole	Paternal dispermy
70,XXY,+16	29	39	20	Partial mole (missed abortion)	Noninformative; tetrasomy 16 noninformative
70,XXX,+18	30	32	18	Partial mole (missed abortion)	Noninformative
68,XX*	32	34	8	Intact sac with fetus	Maternal second meiotic division
68,XX	32	33	10	Ruptured sac	Maternal second meiotic division
68,XXX,−18 (twin 46,XX)	32	33	14	Fragmented tissue	Paternal dispermy
Mean ages	32.1	35.3	13.9		

*Referred from regional hospital in 1976. Reported[7] as Case b.

were eight triploids; in six of these the other abortus was an aneuploid. Warburton et al.[20] suggested that recurrence of triploidy may be higher than expected. These data along with the many aneuploid triploids described above suggest the presence of some common etiologic factor for numerical abnormalities and raise the question of prenatal diagnosis for subsequent pregnancies.

Radiation histories. Among 94 mothers interviewed, 46% gave a history of exposure to preconception abdominal x-ray examinations. This frequency was significantly higher than the 22% recorded among a random series of 217 women of comparable age. The diagnostic procedures consisted mainly of gastrointestinal series, intravenous pyelograms, salpingograms, and lumbar spinal studies. The frequency of similar exposures, salpingograms excepted, among 89 fathers interviewed was 56%. Twenty-eight men reported lower back examinations. Many men in the Hamilton area are employed in heavy industries where back injuries are a hazard and spinal x-ray examinations are often required for insurance purposes before employment. An association between triploidy and radiation exposure has been reported by others.[9, 21] A more detailed analysis of this aspect will be reported.

Comment

Among several reported series of spontaneous abortions, lack of agreement on a number of issues pertaining to triploidy is probably due to the small sample sizes available for analysis. The fairly large series of triploids with known source of parental origin described here may help to resolve some of the differences. It is agreed that parental age is not important; however, our series provides good evidence that when aneuploidy is superimposed on triploidy, maternal age is significantly raised. All reports agree that the main source of the extra chromosome complement is dispermy. Among triploids of maternal origin the timing of the meiotic error is still controversial; in contrast to other reports, our series indicates that retention of the second polar body is not a negligible factor. Perhaps when larger series become available, first and second meiotic errors may be found to occur with equal frequency. Recurrence of triploidy has been recognized, but our data provide evidence of an increased frequency of aneuploidy in triploid sibships. The extent of that increase needs to be further defined in order to be of relevance to prenatal diagnosis. Exposure of the mother to preconception abdominal radiation appears to be of some significance in the etiology of triploidy.

Addendum

Since submission of this report, a second triploid was aborted by a mother included in the above series. Both triploids were 69,XXY. The maternal ages were 22 and 23 years and the paternal ages were 24 and 26 at the time of two abortions.

We gratefully acknowledge the assistance of the obstetric, nursing, pathology, and technical staffs of the Hamilton and regional hospitals in collecting and sending us the specimens used in this analysis. We are also indebted to many medical practitioners who referred the parents to us and many technologists who assisted in the chromosome analyses.

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Breast aspiration biopsy with multihole needles for histologic and cytologic examination

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Multihole needle biopsy has been performed on 166 patients. Commercially available 22-, 20-, 18-, and 16-gauge hypodermic needles (40 to 75 mm in length) are prepared with three sharp-edged holes around the distal part of the needle, allowing aspiration of 3 to 6 ml or more of tissue, for both histologic and cytologic examination. Of patients with carcinoma, fibrocystic disease, fibroadenoma, intraductal proliferation (papillomatosis), and duct ectasia, multihole needle cytologic examination is significantly more accurate (35% to 83%) than single-hole needle examination (11% to 67%). Multihole needle histologic examination, however, is far more accurate, with diagnostic success ranging from 75% to 94% for the disorders described above. The procedure has several uses. For patients with isolated fibrocystic areas, duct ectasia, or papillomatosis, multihole needle biopsy not only is diagnostic but may also be therapeutic by virtue of removal of abnormal tissue by aspiration. Biopsies of areas of microcalcification can be obtained under x-ray control. In patients at high risk of breast cancer (carcinoma in mother and/or sister), breast aspiration is performed when the slightest suspicion arises. In patients with unilateral breast cancer at high risk of bilateral carcinoma (premenopausal breast cancer, lobular carcinoma, tubular carcinoma, family history of breast cancer), random needle biopsy is performed in the contralateral breast for cancer detection. Furthermore, during follow-up of breast cancer patients, biopsies of locoregional changes or suspicious areas in the contralateral breast are obtained with the multihole needle for diagnostic evaluation. Thus multihole needle biopsy represents an improvement over the single-hole needle currently used, with enough tissue provided for adequate initial diagnosis and follow-up diagnostic evaluations in patients with benign and malignant breast disease. (AM J OBSTET GYNECOL 1985;151:70-6.)

Key words: Multihole needle, breast aspiration, histology

In recent years, needle aspiration biopsy has been used extensively for breast cancer screening; it is considered the first invasive diagnostic step in suspected malignancy. Aspiration biopsy is credited with a 50% to 90% accuracy for the cytologic diagnosis of breast cancer.[1-7] According to Hindle and Navin,[8] fine-needle aspiration biopsy has a 75% diagnostic accuracy when a breast tumor is palpable.

A disadvantage of the conventionally used single-hole fine needle (gauges 20 to 23) is the small amount (few drops) of material obtained for cytologic examination. In addition to glandular-epithelial tissue (premenopausal women, 10% to 20%; postmenopausal women, 3% to 5%), the breast contains predominantly fat (50% to 70%) and connective tissue (30% to 40%).[9] Therefore, it is often difficult to obtain epithelial cells for diagnostic evaluation. The rate of inconclusive aspirates, that is, cell-free and unsatisfactory (cell-poor) smears, is 20% to 40%.[3, 4]

Although breast cysts and inflammatory processes can be diagnosed confidently by fine-needle aspiration biopsy, the detection of carcinomas containing relatively large areas of fibrous components is problematic. A scirrhous carcinoma may consist mainly of fibrotic tissue interspersed with cancer cells. Also, in patients with benign breast disease, aspiration of ductal-epithelial elements for diagnostic evaluation and treatment follow-up is difficult because of the predominance of fibrous tissue and fat.

To improve the accuracy of diagnosis of benign, premalignant, and malignant breast neoplasia, multihole biopsy needles were designed with the objectives: (1) to be able to aspirate sufficient amounts of tissue not only for cytologic evaluation but also for histologic examination, thereby reducing the need for surgical biopsy, and (2) to facilitate follow-up of medical treatment of patients with benign and malignant breast disease.[10, 11]

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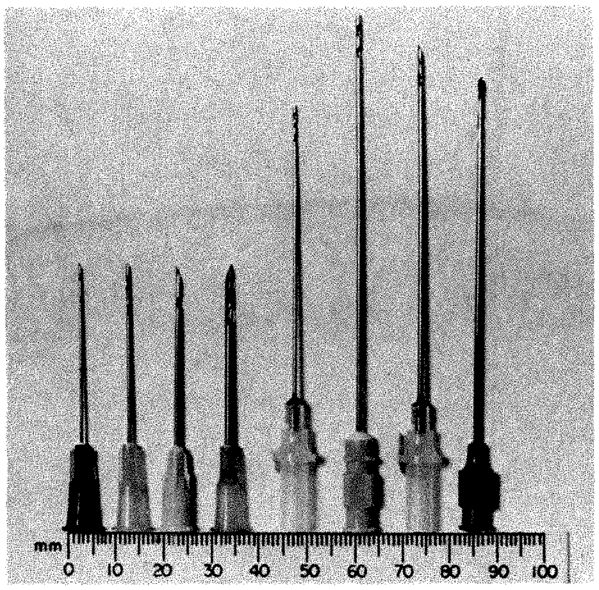

Fig. 1. Multihole biopsy needles. The 16-, 18-, 20-, and 22-gauge needles (40 to 75 mm in length) with three sharp-edged holes at their distal parts are depicted.

Material and methods

Sizes and preparation of multihole needles. Commercially available 22-, 20-, 18-, and 16-gauge hypodermic needles (40 to 75 mm in length) are prepared with three sharp-edged holes along the distal part (Fig. 1). The three holes are staggered around the needle within 12 to 15 mm of its tip and are made by a grinding wheel 1.7 mm wide. Grinding with the edge of the wheel is performed perpendicular to the needle shaft. Under magnification, small pieces of ground metal are removed with a sharp knife from the edges of the needle holes. The edges are made as sharp as possible. In 22-, 20-, 18-, and 16-gauge needles, the respective diameters of prepared ovoid holes are: 0.4 by 1.8 mm, 0.6 by 2.3 mm, 0.8 by 2.8 mm, and 1.1 by 3.8 mm. The holes are so distributed over the distal shaft of the needle that enough stability is maintained for insertion of the needle through the skin into breast tissues for aspiration.

Indications and aspiration technique. Needle aspiration biopsy is indicated in patients with a breast mass (cyst), a lumplike structure, a hard, dense area, or any abnormal tissue area, as defined by clinical examination, mammography, or sonography. In patients at extraordinarily high risk of breast cancer, needle aspiration is performed when the slightest suspicion arises. The technique of multihole needle aspiration is similar to that performed with a conventional single-hole needle. After skin disinfection with an antimicrobial agent such as chlorhexidine gluconate (Hibiclens) and local 1% lidocaine infiltration, the breast mass or suspicious area to be sampled is fixed between thumb, index finger, and middle finger. After the needle (attached to a 30 ml disposable syringe) is inserted, approximately 10 needle passages back and forth in various directions and additional rotating movements are performed under negative pressure within the mass or suspicious tissue area (Fig. 2). Thereby tissue sucked onto or into the needle holes is cut off by the sharp edges and can be aspirated. In patients with a small tumor or a mass of hard consistency, a 22- or 20-gauge multihole needle is used first, followed when possible by an 18-gauge needle inserted through the previous skin puncture. In patients with breast tissues consisting mainly of connective (fibrous) tissue and fat (fibrocystic disease, postmenopausal), suspicious areas are aspirated with an 18-gauge needle and, if more material is desired, with a 16-gauge needle. To prevent loss of aspirated tissues, the needle is removed under maintained negative pressure. Afterward, the aspiration area is covered with a sterile 10 by 10 cm gauze sponge, and sustained pressure is applied by means of a 5-pound bag for at least 20 minutes to minimize hematoma formation.

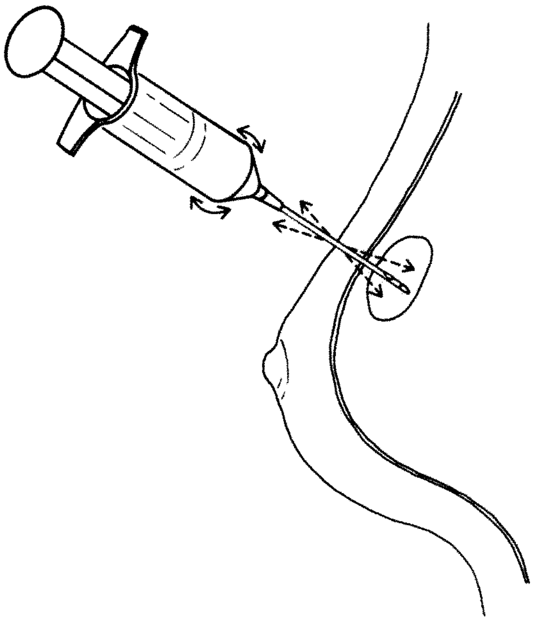

Fig. 2. Breast aspiration. The multihole needle attached to a 30 ml disposable syringe is inserted into the mass and negative pressure is applied by pulling the plunger back. By means of the syringe the needle is moved back and forth in various directions and additional rotating movements are performed within the mass for aspiration of tissue. (From Vorherr H. Breast aspiration biopsy. Am J Obstet Gynecol 1984;148:128.)

Usually 3 to 6 ml of material is aspirated. In patients

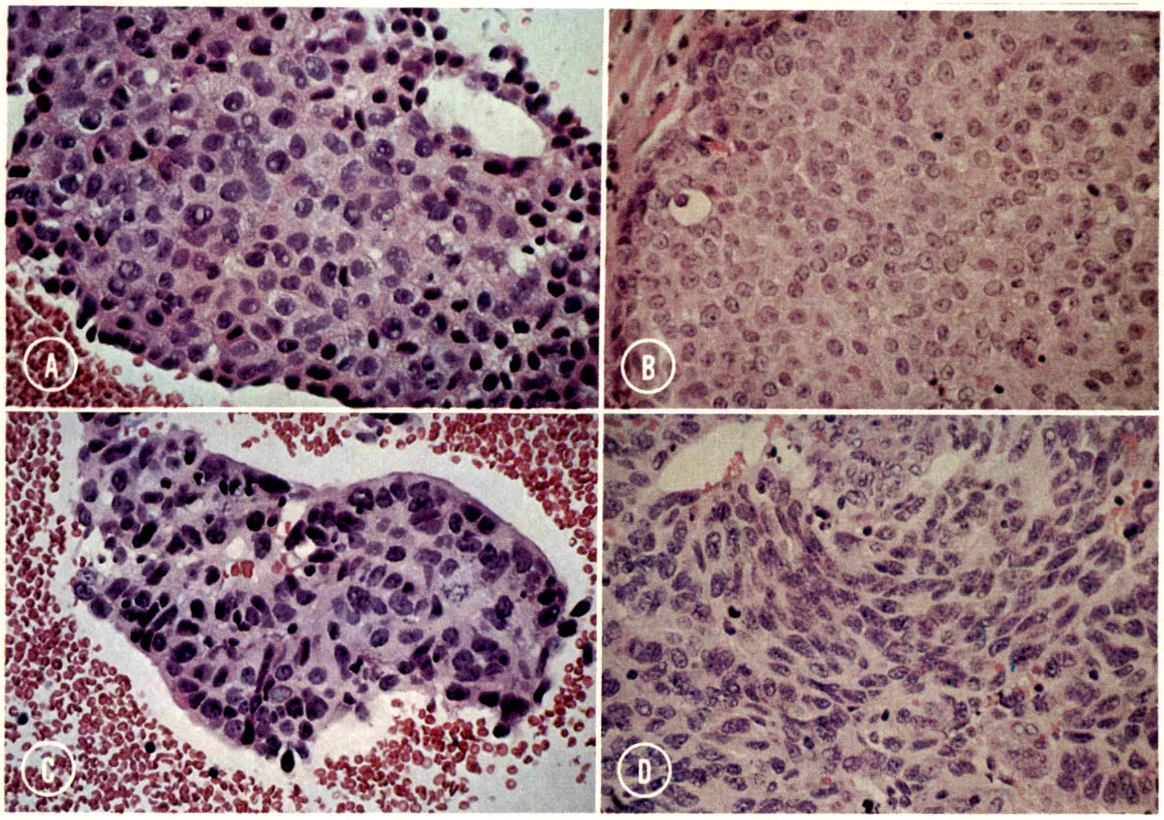

Fig. 3. Histologic diagnosis of breast cancer by multihole needle aspiration and surgical biopsy. *A* and *B*: Histologic features of a 29-year-old patient with invasive ductal carcinoma. *A:* Multihole needle aspiration; *B:* surgical biopsy. *C* and *D:* Histologic features of a 44-year-old patient with invasive ductal carcinoma. *C*: Multihole needle aspiration; *D*: surgical biopsy. (Original magnification ~400-fold.)

Table I. Diagnostic accuracy of needle aspiration in 166 patients with benign and malignant breast disease

Diagnosis	*Single-hole needle cytologic examination (%)*	*Multihole needle*	
		Cytologic examination (%)	*Histologic examination (%)*
Carcinoma (n = 15)	67	73	93
Fibrocystic disease (n = 86)	31	47	94
Fibroadenoma (n = 26)	58	83	92
Intraductal proliferation (papillomatosis) (n = 12)	11	67	75
Duct ectasia (n = 27)	14	35	88

with pronounced duct ectasia and secretory retention, ≥8 ml of yellowish material is obtained. The more solid particles of the aspirate are ejected on lens paper which is folded up into a bag and then immersed into formalin for histologic examination. The remaining more liquid material is squirted on a glass slide for cytologic evaluation.

Evaluation of the aspirate. The aspirate usually contains erythrocytes, fat, connective tissue, and ductal-alveolar epithelium. The evaluation of the aspirate considers epithelial elements, fibrous changes, fibroblast activity, and inflammatory reactions.

Malignant cells or cells suspected to be carcinoma are distinguished by nuclear pleomorphism, hyperchromatism, loss of cohesiveness, and an increased rate of mitosis (Fig. 3). In patients with fibrocystic disease, cohesive ductal epithelium with round or oval small and slightly hyperchromatic nuclei and a scant rim of cytoplasm, often accompanied by apocrine metaplasia, are typical features. Intraductal or intracystic epithelial

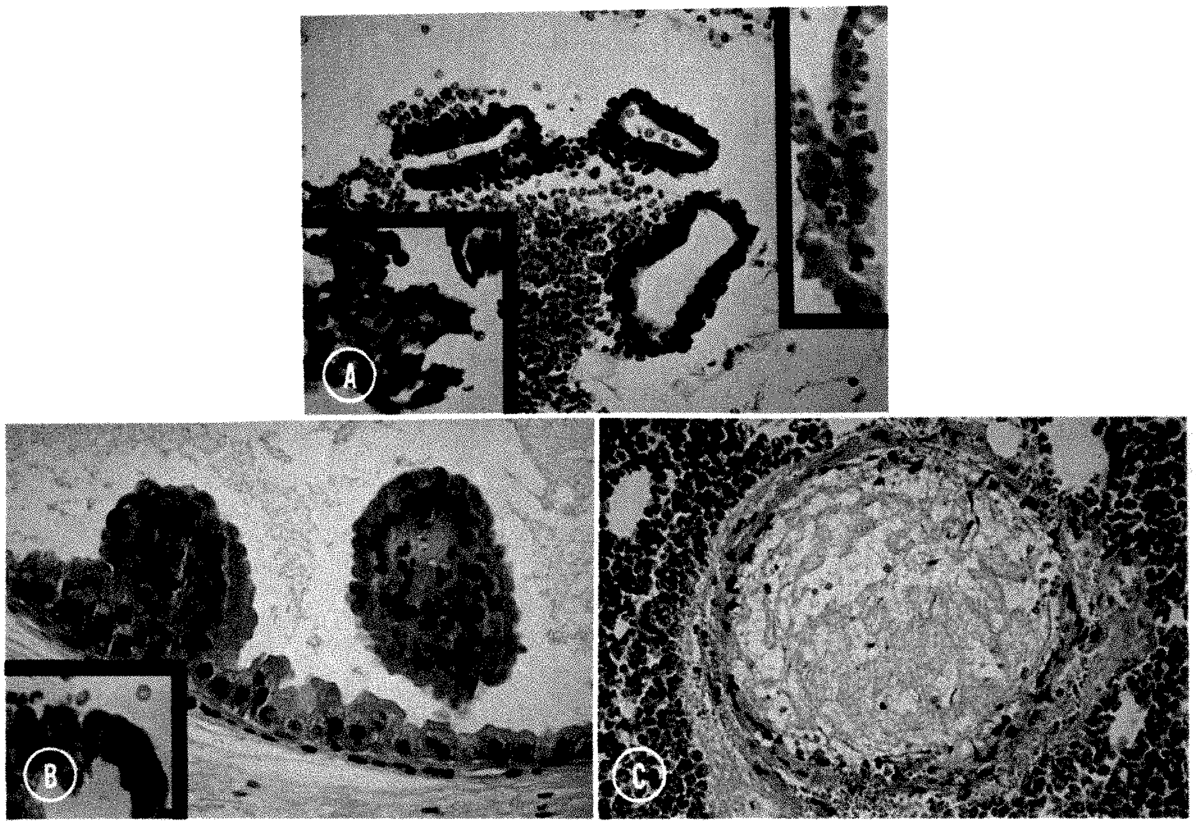

Fig. 4. Diagnosis of benign breast disease by multihole needle aspiration. *A:* Fibrocystic breast disease in a 37-year-old patient showing ductal structures with relatively small epithelial cells and nuclei and some intraductal proliferation with slight nuclear atypia *(left)* and apocrine metaplasia *(right)*. *B:* Intracystic proliferation and apocrine metaplasia *(left)* in a 44-year-old patient with fibrocystic breast disease. *C:* Estrogen-induced fibrocystic breast disease in a 56-year-old patient showing a microcyst and pericystic fibrosis. (Original magnification ~400-fold.)

proliferation (typical or atypical) may be found (Fig. 4). Typical for fibroadenoma are numerous epithelial strands in a tubular-lobular arrangement. The nuclei of epithelium of fibroadenomas are enlarged (8 to 10 μm in diameter), vary in size, and are moderately hyperchromatic. Often apocrine metaplasia coexists and lactation-like areas may be observed (Fig. 5). The histologic features of aspirates of patients with intraductal proliferation (papillomatosis) show large polygonal cells with a clear rim of cytoplasm and large round or oval nuclei; the cytoplasm may be eosinophilic and in transition to apocrine metaplasia (Fig. 6). In patients with duct ectasia a lipidlike material is aspirated in larger quantities (6 to 10 ml); ductal epithelium is usually sparse. In patients with complicated duct ectasia periductal round cell infiltration is observed (Fig. 7).

Results

In Table I, the results of multihole needle aspiration biopsy in 166 patients are presented. In only one of the 15 patients with cancer, aspiration histologic examination failed to reveal malignant cells. Whenever epithelial elements are obtained, the diagnostic value (accuracy) of aspiration histologic examination is equal to that of surgical biopsy (Fig. 3). In patients with questionable axillary lymph nodes, multihole needle aspiration is also a successful diagnostic procedure (Fig. 8).

Also, in women with benign breast disease, aspiration histologic evaluation is more accurate than cytologic examination (Table I). Even though multihole needle cytologic examination is superior to single-hole needle cytologic examination, multihole needle aspiration histologic evaluation is by far more accurate (Table I).

In almost every patient, hematoma (sizes vary from 1 by 2 cm to 4 by 5 cm in diameter) is observed at and around the aspiration site. In all patients hematomas resolved spontaneously within 2 to 4 weeks. Patients are instructed to leave the pressure dressing on for 24 hours and in case of pain, swelling, and/or fever to call the physician. Continued bleeding or infection was not observed in any of the patients examined.

Comment

Aspiration histologic examination is preferable to cytologic examination because the diagnostic quality of

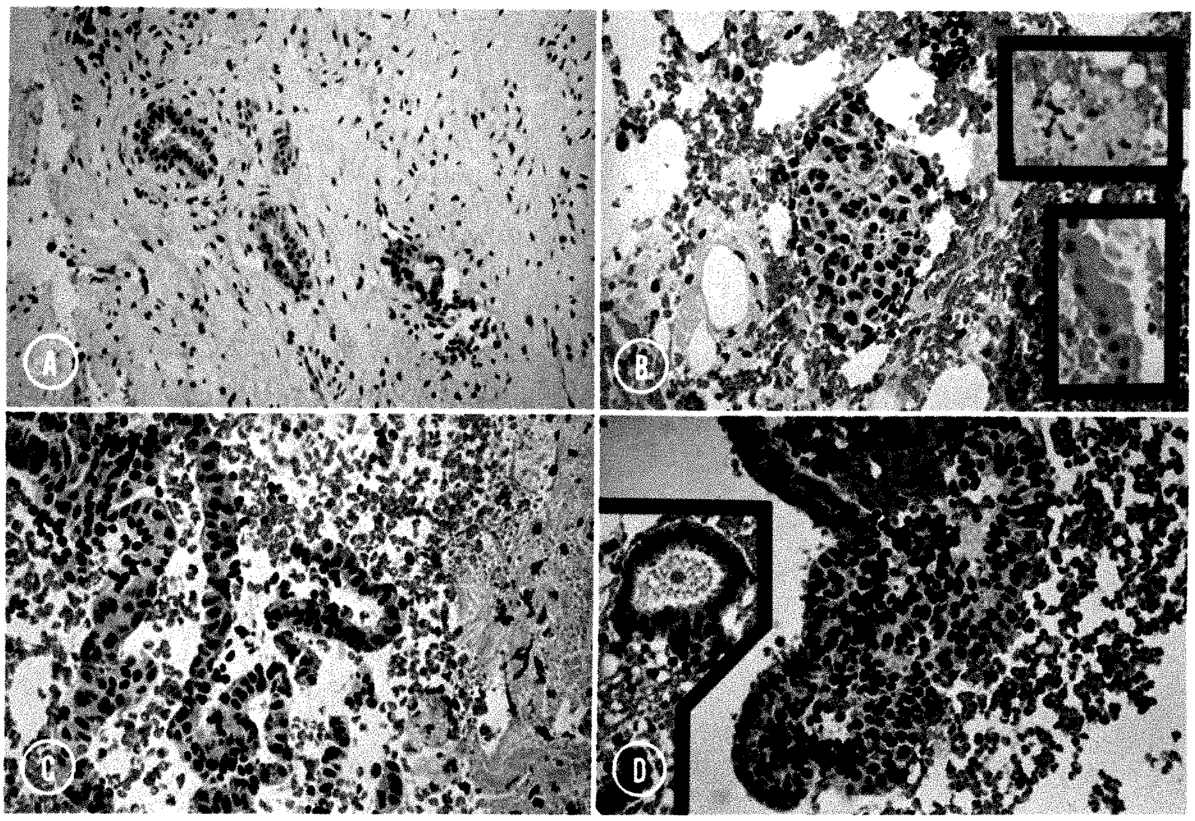

Fig. 5. Fibroadenoma diagnosed by multihole needle aspiration. *A*: Fibroadenoma of a 21-year-old patient showing glandular structures within fibrous tissue. (Original magnification ~200-fold.) *B*: Larger original magnification (~400-fold) of *A* showing epithelial elements of fibroadenoma (enlarged nuclei, variation in nuclear size, hyperchromatism), fibrous components *(upper right)*, and apocrine metaplasia *(lower right)*. *C*: Same patient as in *A* and *B* showing epithelial strands of fibroadenoma with fibrous components *(right)*. (Original magnification ~400-fold.) *D*: Fibroadenoma of a 25-year-old patient showing epithelial elements of fibroadenoma (enlarged nuclei, variation in nuclear size, hyperchromatism) and ductal morphologic features with lactation-like changes *(left upper part)* and apocrine metaplasia *(left lower part)*. (Original magnification ~400-fold.)

formalin-paraffin–processed and hematoxylin-eosin–stained tissues is superior. Whereas aspirated blood may interfere with cytologic evaluation of the smear, this is not the case with aspiration histologic examination because homogeneous red-stained erythrocytes are contrasted by blue-stained epithelial cells (Figs. 3 to 8). Also, with cell-poor smears uncertainty may arise because some cancer cells may appear benign or some benign ductal cells may be suggestive of malignancy.

Multihole needle aspiration may spare the patient surgical biopsy. In the scheme of Fig. 9, the diagnosis and management of benign and malignant breast disease are outlined. For patients with isolated fibrocystic areas, duct ectasia, or intraductal proliferation, multihole needle aspiration can be therapeutic (Fig. 6).

Multihole needle aspiration is also used in patients at extraordinarily high risk of breast cancer (carcinoma in mother and/or sister) without clinical or mammographic suspicion of a lesion. In these patients, biopsies are obtained when the slightest suspicion arises. Also, in patients with unilateral breast cancer at high risk of bilateral carcinoma (premenopausal breast cancer, lobular carcinoma, tubular carcinoma, family history of breast cancer), we perform random multihole needle aspiration of the contralateral breast. During patient follow-up, biopsies of locoregional changes or suspicious areas in the contralateral breast are obtained with the multihole needle. Because needle aspiration leaves no scarring, future diagnostic examinations (clinical, mammography) are not impaired as is the case with scarring following surgical biopsy.

The assistance of Dina Reid, R.N., C.O.G.N.P., Coordinator of the University of New Mexico Hospital Breast Clinic, is herewith acknowledged. The preparation of multihole needles by Raymond Klein, technical engineer, is appreciated.

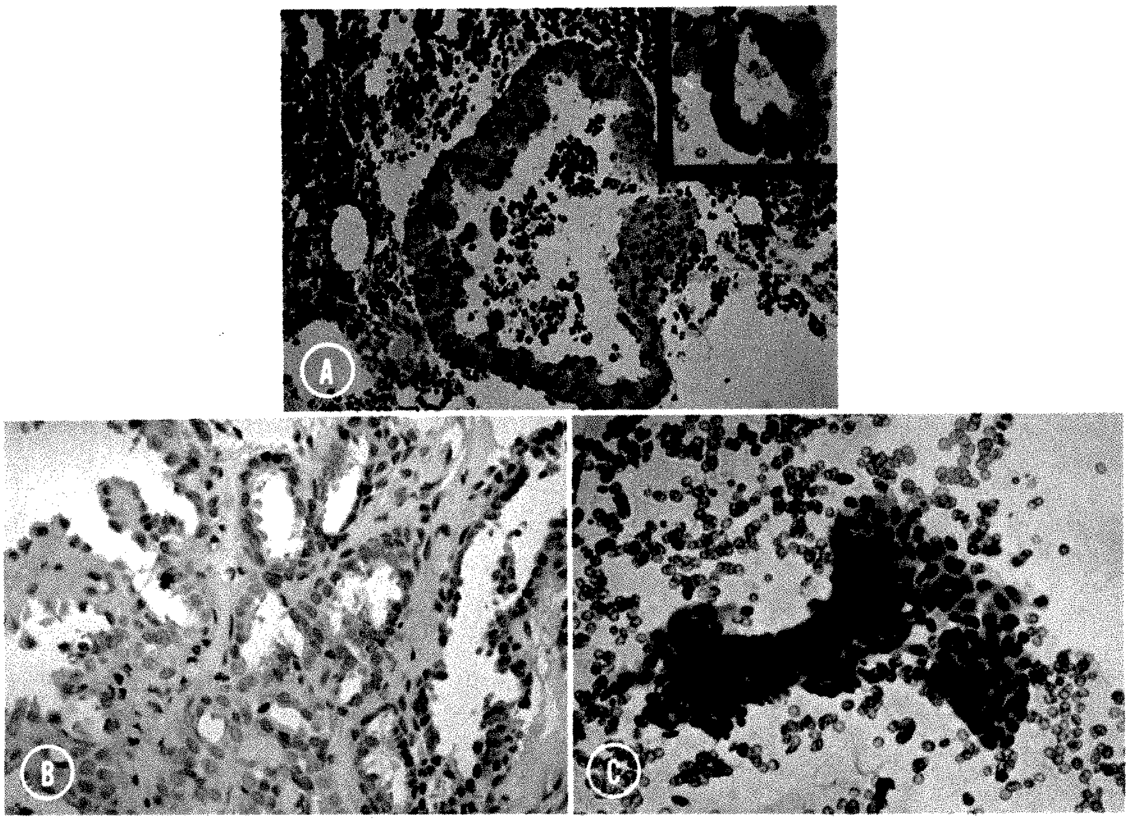

Fig. 6. Diagnosis of intraductal proliferation (papillomatosis) by multihole needle aspiration. *A:* Intraductal proliferation (papillomatosis) (original magnification ~200-fold) and apocrine metaplasia *(right upper corner)* (original magnification ~400-fold) in a 37-year-old patient with fibrocystic breast disease. *B:* Papillomatosis (aspiration histologic examination) in a 57-year-old patient with bloody nipple discharge. In this patient multihole needle aspiration was therapeutic. (Original magnification ~400-fold.) *C:* Aspiration cytologic examination of patient in *B* showing a large cluster of papilloma cells and erythrocytes. (Original magnification ~400-fold.)

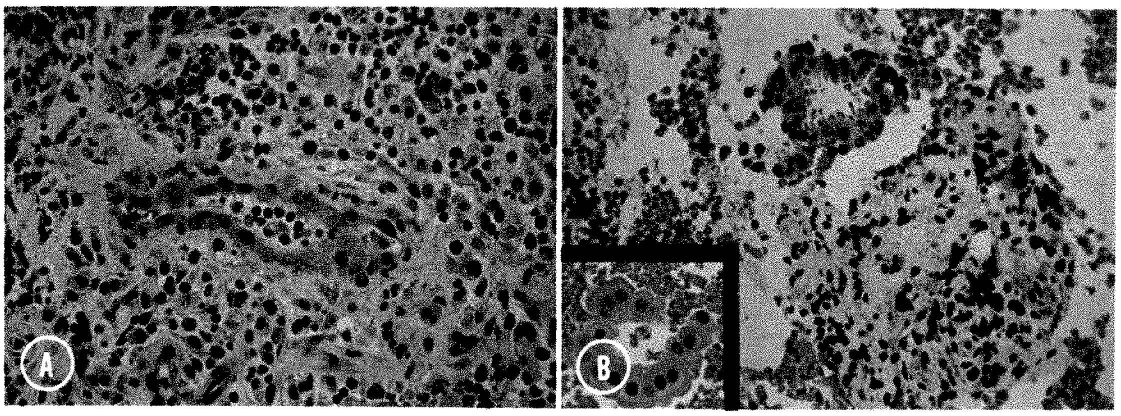

Fig. 7. Diagnosis of duct ectasia and periductal round cell infiltration by multihole needle aspiration. This 45-year-old patient had clinical symptoms of fibrocystic breast disease. *A:* Periductal round cell infiltration. *B:* Ductal structure with adjacent area of fibrosis and necrosis and apocrine metaplasia *(left)*. (Original magnification ~400-fold.)

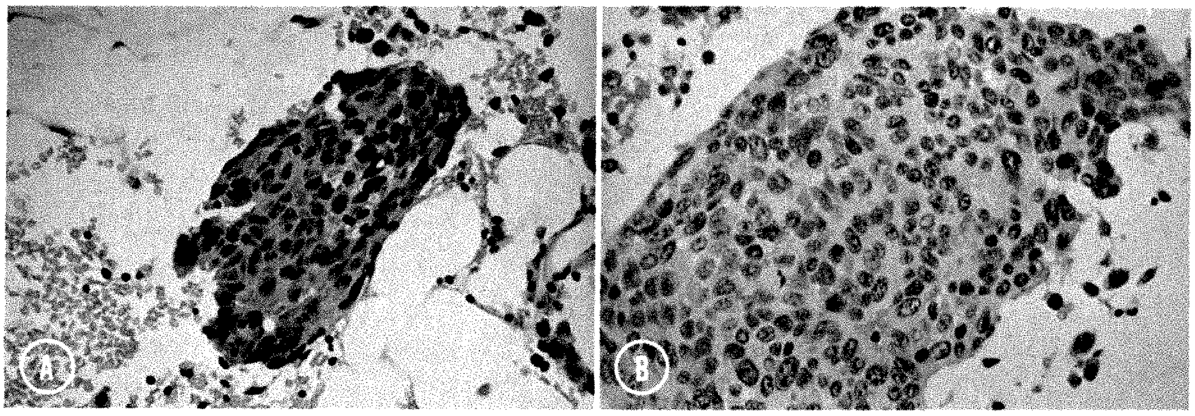

Fig. 8. Histologic diagnosis of axillary lymph node metastasis. *A:* Multihole needle aspiration of a clinically positive axillary node. *B:* Surgical excision of a clinically positive axillary node. (Original magnification ~400-fold.)

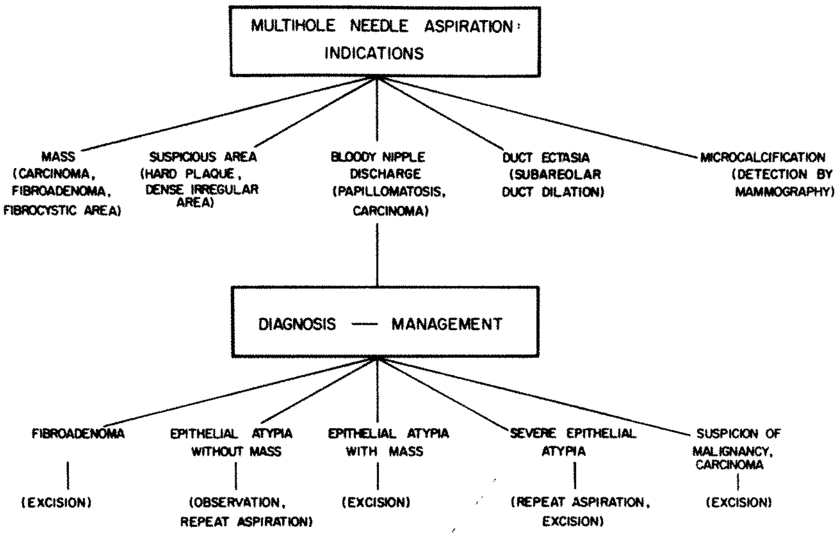
MULTIHOLE NEEDLE ASPIRATION: INDICATIONS
MASS (CARCINOMA, FIBROADENOMA, FIBROCYSTIC AREA)
SUSPICIOUS AREA (HARD PLAQUE, DENSE IRREGULAR AREA)
BLOODY NIPPLE DISCHARGE (PAPILLOMATOSIS, CARCINOMA)
DUCT ECTASIA (SUBAREOLAR DUCT DILATION)
MICROCALCIFICATION (DETECTION BY MAMMOGRAPHY)
DIAGNOSIS — MANAGEMENT
FIBROADENOMA
(EXCISION)
EPITHELIAL ATYPIA WITHOUT MASS
(OBSERVATION, REPEAT ASPIRATION)
EPITHELIAL ATYPIA WITH MASS
(EXCISION)
SEVERE EPITHELIAL ATYPIA
(REPEAT ASPIRATION, EXCISION)
SUSPICION OF MALIGNANCY, CARCINOMA
(EXCISION)

Fig. 9. Multihole needle aspiration for diagnosis and management of breast disease. In patients with isolated fibrocystic areas, intraductal proliferation (papillomatosis), microcalcification, and duct ectasia, multihole needle aspiration is diagnostic but can also be therapeutic because the abnormal tissue can be removed by aspiration. Fibroadenoma requires surgical excision. When the aspirate shows carcinoma, the patient is informed about the nature of the disease, and further diagnostic steps and treatment alternatives are discussed.

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A Near-Spotless Record.

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Total Cycles‡	4.2%	2.9%

Lo/Ovral® offers less breakthrough bleeding and spotting, along with high contraceptive efficacy, at a low dose—just 30 mcg ethinyl estradiol and 0.3 mg norgestrel.

LOW DOSE

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[1] Dickey RP: Managing Contraceptive Pill Patients, 3rd ed., Durant, Oklahoma, Creative Infomatics, Inc., 1983.

*Rates are derived from separate studies from different investigators in several population groups and cannot be compared precisely.

†Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.

‡Data on file for 22,489 total cycles, Wyeth Laboratories. **See important information on following page.**

IN BRIEF:

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000, ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

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6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic), mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

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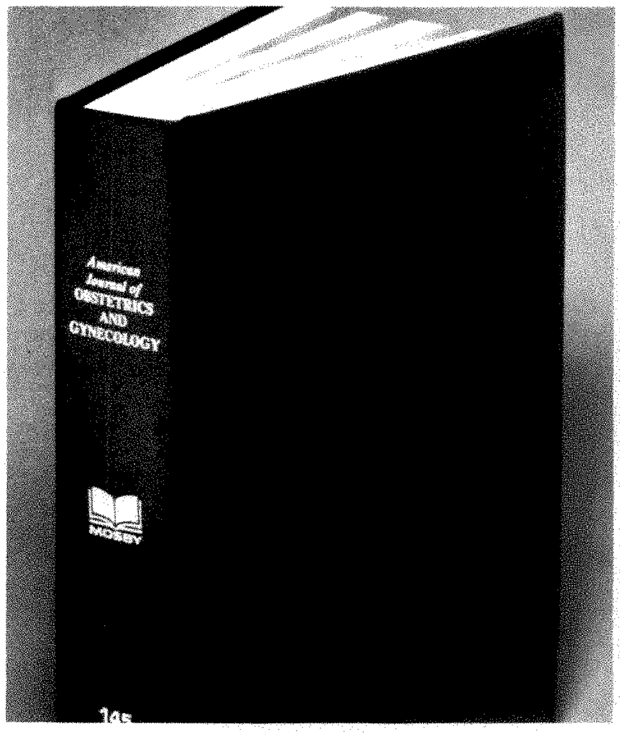

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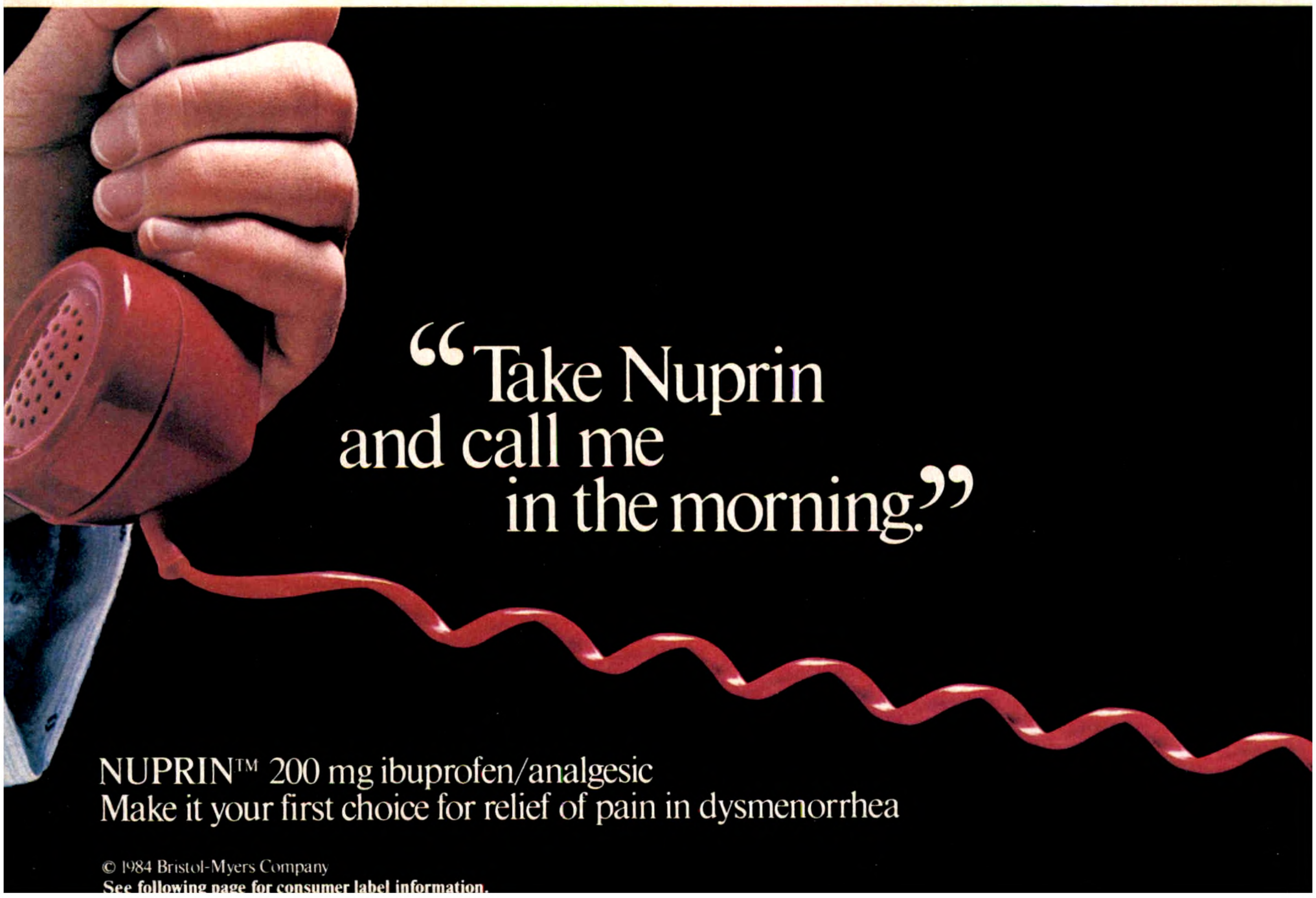

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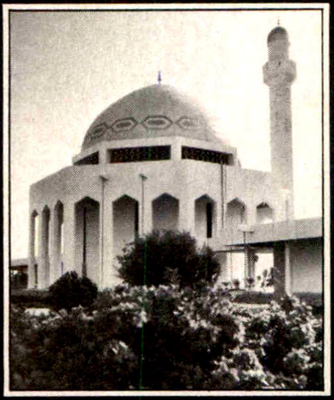

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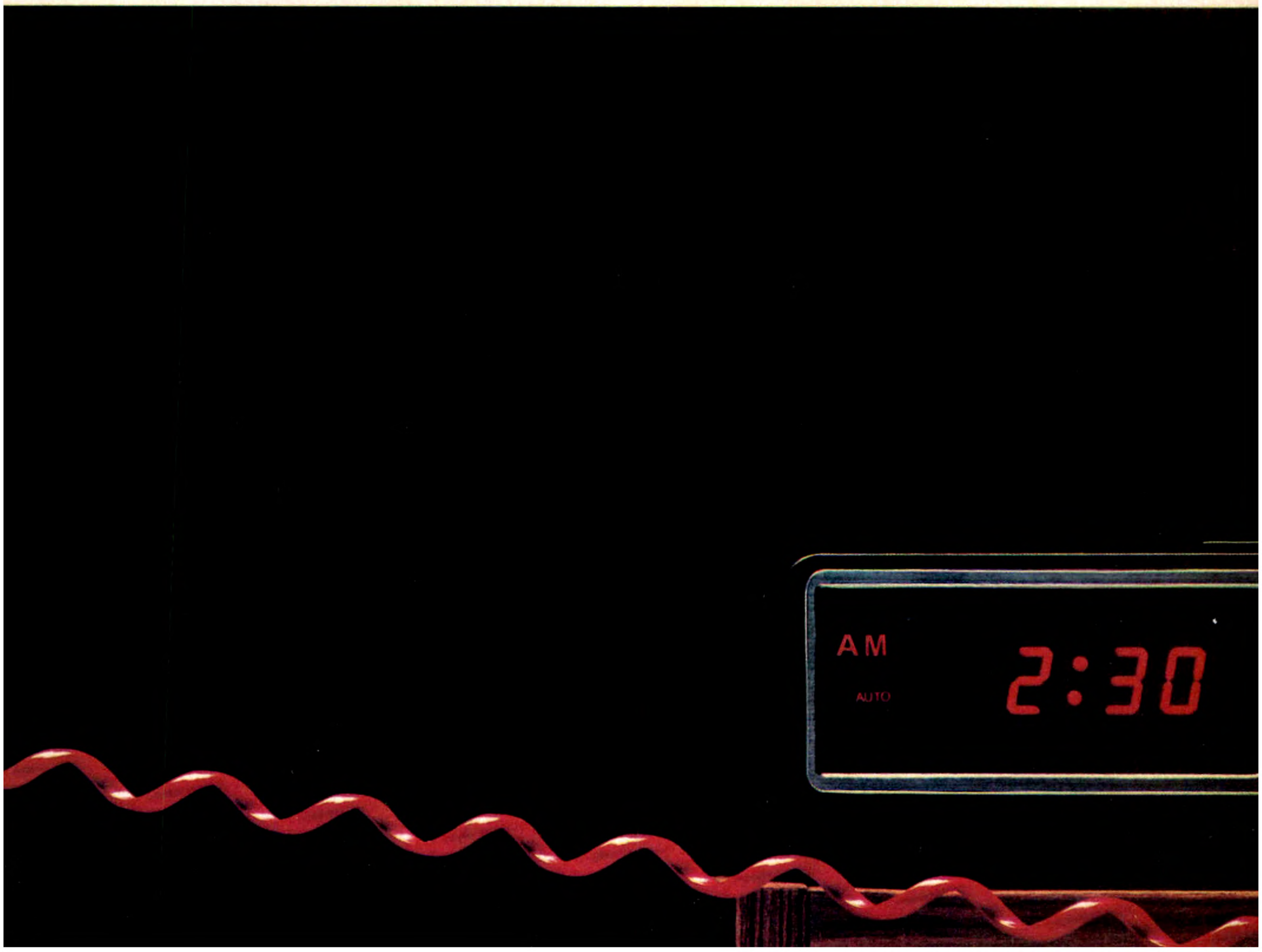

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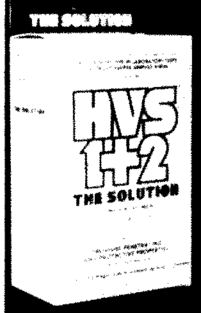

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The psychological impact of false positive elevations of maternal serum α-fetoprotein

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Winston-Salem, North Carolina

The purpose of this study was to assess the psychological impact of maternal serum α-fetoprotein screening on pregnant women with false positive elevations of maternal serum α-fetoprotein and on their male partners. Subjects with maternal serum α-fetoprotein elevations and their partners exhibited significantly heightened anxiety as measured by the State-Trait Anxiety Inventory following notification of the elevation, and this anxiety persisted until normal results were obtained by further testing. Heightened anxiety was not alleviated by counseling prior to definitive testing. Once normal results were obtained, anxiety returned to control levels and heightened anxiety was not observed throughout the remainder of pregnancy. Maternal serum α-fetoprotein elevations had little effect on maternal attitudes toward pregnancy as measured by the Maternal Attitudes to Pregnancy Inventory. Similarly, no differences were observed between subjects with normal and elevated maternal serum α-fetoprotein levels with regard to depression, marital discord, work attendance, or work productivity. (AM J OBSTET GYNECOL 1985;151:77-82.)

Key words: α-Fetoprotein screening, neural tube defects, anxiety, pregnancy

Neural tube defects, including anencephaly and spina bifida, are among the most common and most severe congenital malformations. In the United States, they occur with a frequency of 1 to 2 per 1000 live births.[1] The vast majority of fetal neural tube defects can be accurately diagnosed prenatally using the combination of ultrasonography and measurement of amniotic fluid α-fetoprotein and acetylcholinesterase.[2] Prenatal diagnosis is commonly offered to couples known to be at increased risk by virtue of a positive family history. It is important to note in this regard, however, that over 90% of affected infants are born to women with no previous family history of similar defects. Therefore, prenatal diagnosis of the majority of neural tube defects could never be achieved by reliance on family history as the sole screening tool.

In 1977 a 19-center collaborative study from the United Kingdom documented the effectiveness of maternal serum α-fetoprotein screening in selecting pregnant women from the population at large for whom prenatal diagnosis for neural tube defects was warranted.[3] Subsequently, several large studies in the United States have demonstrated the effectiveness and feasibility of maternal serum α-fetoprotein screening in this country.[4-6] These developments have generated a great deal of debate regarding the advisability of including maternal serum α-fetoprotein screening in routine obstetrical care. Much of the concern of those opposed to widespread screening has centered on the potential adverse psychological impact of screening on the pregnant woman.[7, 8] In particular, concern has been repeatedly expressed about the impact of so-called false positive maternal serum α-fetoprotein elevations. Since 3% to 7% of all pregnant women undergoing screening will have at least one maternal serum α-fetoprotein elevation and only a small fraction of these will be associated with a fetal neural tube defect, it is critically important that this issue be addressed.

The present study was designed to measure the psychological impact of false positive maternal serum α-fetoprotein elevations on the pregnant woman, her male partner, and the mother-infant pair. The data presented here relate to the psychological response of the woman and her partner; data relating to the mother-infant relationship are still being collected.

Material and methods

Prescreening assessments of anxiety and attitudes toward pregnancy. Subjects in this study were participants in a screening program for neural tube defects in North Carolina. Details regarding this screening program have been previously published.[4, 9] Between October 15, 1981, and March 15, 1983, all women presenting for screening were asked to participate in the

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Table I. Characteristics of study groups (female)

		Experimental groups			
	Control subjects	*1*	*2*	*3*	*Difference**
No. of subjects	192	70	42	52	—
White (%)	84	77	69	75	NS
Primigravid (%)	55	43	57	59	NS
Mean age (yr)	28	28	28	27	NS
Planned pregnancies (%)	74	61	54	63	NS
Hollingshead scale (% in each class)					p<0.01
I	17	5	13	11	
II	28	18	21	13	
III	29	37	26	28	
IV	22	37	26	35	
V	4	3	13	13	

*Difference between experimental groups and control subjects as determined by χ^2 analysis.

baseline (pre-screening) phase of the study; 6084 of 6404 patients (95%) agreed. The baseline assessment consisted of two questionnaires: the State-Trait Anxiety Inventory[10] and the Maternal Attitude to Pregnancy Instrument.[11] The State-Trait Anxiety Inventory consists of a 20-item A-State scale, designed to assess the level of relatively transient situation-related (state) stress perceived in a particular situation, and a 20-item A-Trait scale, designed to measure the relatively stable long-term "resting level" of anxiety in the individual. The State-Trait Anxiety Inventory has been subjected to intensive validity and reliability testing and is suitable for repeated administrations. The Maternal Attitude to Pregnancy Instrument consists of 48 items with which the respondent must strongly agree, disagree, or strongly disagree. It is designed to assess attitudes toward pregnancy along several dimensions, including fearfulness, attitudes toward child, attitudes toward doctor, concern over child's normalcy, and general well-being. It has not been as extensively evaluated as the State-Trait Anxiety Inventory with regard to its suitability for repeated administrations. The questionnaires were completed before blood was drawn for maternal serum α-fetoprotein determination. Women who agreed to participate were given a packet to take home to their husbands or partners which contained a consent form, the State-Trait Anxiety Inventory, and a stamped, addressed envelope. Male participants were asked to complete and return the questionnaires within 24 hours; 3237 (53%) were returned.

Continuing assessments

Experimental groups. All women who had a maternal serum α-fetoprotein elevation on the initial sample were notified by telephone within 1 week and were asked to come in for a repeat assessment. All subjects who returned to have a second sample drawn and their husbands or partners were asked to participate in the continuing phase of the study; 179 of 205 women (87%) and 121 of their partners (59%) agreed. After the explanation of the study but before the blood was drawn, women were given the A-State scale and Maternal Attitude to Pregnancy Instrument. If present, men were given the A-State scale. Otherwise, it was sent home with the female partner.

Seventy of the 179 participating experimental subjects had a normal maternal serum α-fetoprotein level on the second determination. These subjects constitute group 1. They were notified of their normal results by telephone within 1 week and a home visit was conducted the following day to administer the A-State scale and Maternal Attitude to Pregnancy Instrument to the woman and the A-State scale to her male partner. A general information questionnaire providing demographic data was also completed at that time.* In order to equate the number of test administrations and intertest intervals across each group, two additional home visits were made to this group at weekly intervals, with the same questionnaires being given each time. Therefore, including the baseline assessment, five measures of state anxiety and attitudes toward pregnancy were obtained over approximately a 5-week period. Thereafter, similar assessments were conducted by mail at 26, 30, and 34 weeks' gestation. At 37 to 38 weeks a home visit was conducted to administer the usual questionnaires and an additional questionnaire relating to symptoms of depression, changes in the marital relationship, number of contacts with physician, work attendance, and work productivity. The latter questionnaire was developed specifically for this study.* Subjects were asked to call as soon as possible after delivery or to mark delivery date on a postcard provided to them and return it. Subjects not heard from 1 week after the due date were called weekly to determine when delivery occurred.

Data on outcome of pregnancy were routinely obtained from hospital delivery records. In addition, sub-

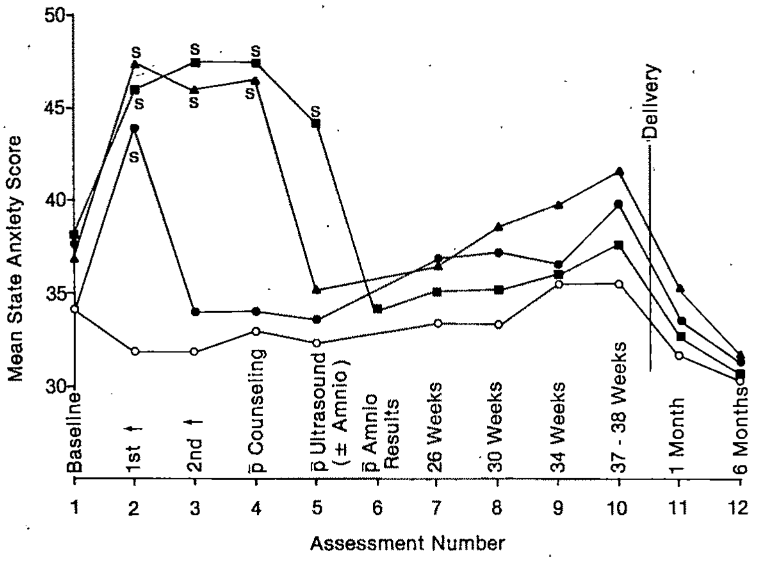

Fig. 1. STAI A-State scores in experimental and control subjects. ○–○, Controls; ●–●, group 1; ▲–▲, group 2; ■–■, group 3; *s*, point at which the plotted value is significantly different from control; $p < 0.01$; p̄, after.

jects were sent a questionnaire by mail relating to labor and delivery and complications occurring in mother and/or infant.* At 1 month of age, infants were seen for an evaluation. At this time, the A-State scale was again administered to both parents. When the infant reached 6 months of age, the A-State scale was sent to the parents by mail.

All subjects with two sequential maternal serum α-fetoprotein elevations were notified by telephone of the second elevation and asked to come in for a counseling session. Prior to counseling, they were asked to complete the A-State scale and Maternal Attitude to Pregnancy Instrument. Following counseling but prior to ultrasonography (on the same or different days), the questionnaires were again administered. Forty-two subjects were found upon ultrasound examination to have a "benign" explanation for their maternal serum α-fetoprotein elevations (more advanced gestational age or twins). These subjects constitute group 2. One day after notification of the findings of normalcy on ultrasound studies, a home visit was conducted to administer the standard questionnaires and general information questionnaire. Subsequent assessments were conducted as described for group 1.

Fifty-two subjects were found by ultrasonography to have a single fetus of the predicted gestational age and chose to undergo amniocentesis to rule out a fetal neural tube defect. These subjects form group 3. The fifth administration of the A-State scale and Maternal Attitude to Pregnancy Instrument in this group occurred after the amniocentesis was performed but before the results were available. An extra assessment was done on this group by home visit 1 day after telephone notification of the normal amniotic fluid results. Subsequent assessments were as described for group 1.

Four subjects were candidates for amniocentesis but refused the procedure. Therefore, they could not be reassured with regard to the absence of a neural tube defect in the fetus. These subjects formed group 4. A full sequence of assessments was conducted on them but the data will not be presented, since the numbers were too small for meaningful analysis. Seventeen subjects were found to have suffered a fetal death or to have a fetus with a neural tube defect. They were subsequently excluded from the study.

Control subjects. Each time an experimental subject was identified, a potential control subject was identified from among patients screened on the same date at the same site (health department or private hospital) who had a normal maternal serum α-fetoprotein level. An attempt was made to select a control subject matched to the experimental subject for race, age, and parity. If more than one matched subject was available, the control subject was randomly selected from among them. If no matching subject could be identified, a nonmatched control subject was selected. The potential control subjects were contacted by telephone, informed of their normal results, and asked to participate in the continuing phase of the project. When a subject refused, another potential control subject was called. In all, 262 potential control subjects were contacted to ob-

*Copies of the questionnaire may be obtained from the author upon request.

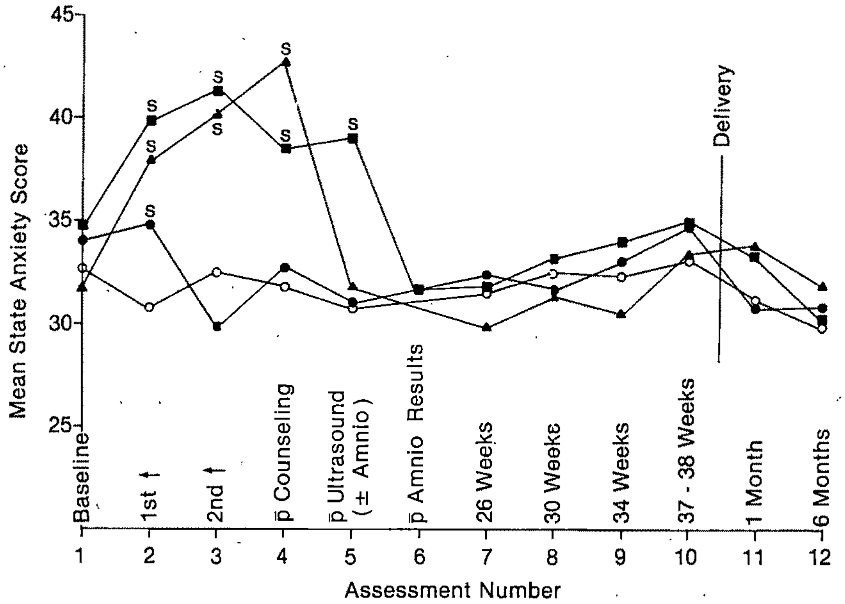

Fig. 2. STAI A-State scores in male partners of experimental and control subjects. ○–○, Controls; ●–●, group 1; ▲–▲, group 2; ■–■, group 3; *s*, point at which the plotted value is significantly different from control; $p < 0.01$; $\bar{p}$, after.

tain 192 participants (73%); 77% of male partners in the control group (148 subjects) agreed to participate.

Four weekly home visits were conducted to administer questionnaires to control subjects, the first being scheduled 1 day after the telephone contact. Subsequent assessments were the same as described for experimental groups.

Results

Characteristics of the control and experimental groups are described in Table I. Significant differences were observed between groups only for socioeconomic status as determined by Hollingshead's two-factor index of social position.[12] Control subjects tended to be better educated and larger numbers were professionals; therefore, more control subjects than experimental subjects were found in the higher social classes (I and II).

State-Trait Anxiety Inventory A-trait and A-state scores and Maternal Attitude to Pregnancy Instrument scores were analyzed by multivariate analysis of variance, controlling for race, social class, and planned versus unplanned pregnancy. Each experimental group was compared at each point in time to the control group. Prior to screening, there was no significant difference in trait or state anxiety between groups of female or male subjects. Similarly, there was no measurable difference in attitudes toward pregnancy between experimental and control groups.

Results of sequential assessments of state anxiety in female subjects are illustrated in Fig. 1. A significant rise in anxiety was observed in all experimental subjects following notification of the first maternal serum α-fetoprotein elevation. If the second maternal serum α-fetoprotein level was normal, anxiety dropped sharply to a level not significantly different from the control level. Subjects who had two successive maternal serum α-fetoprotein elevations maintained significantly elevated levels of anxiety which were not substantially altered by extensive counseling prior to further testing. In subjects found on ultrasound examination to have a benign explanation for the maternal serum α-fetoprotein elevation (twins or more advanced gestational age), anxiety returned promptly to the control level. Subjects who underwent amniocentesis exhibited some reduction in anxiety following the procedure (which followed immediately after ultrasonography), but maintained a significantly elevated anxiety level as compared to control subjects until notification of the normal amniotic fluid results. Once normal results were obtained, there was no evidence of any residual anxiety persisting or recurring throughout the remainder of the pregnancy or for up to 6 months after delivery in any experimental group. All groups exhibited a gradual rise in anxiety as the delivery date approached with a significant fall by 1 month after delivery.

The course of anxiety in male subjects is illustrated in Fig. 2. A similar pattern of heightened anxiety was observed in male partners of experimental subjects during the testing process but again this was completely alleviated by ultimate notification of normal results. No residual increased anxiety was observed.

The sequential assessments of maternal attitudes toward pregnancy are illustrated in Fig. 3. Although scores of experimental groups were generally lower than those of control subjects throughout pregnancy, a significant difference between experimental and con-

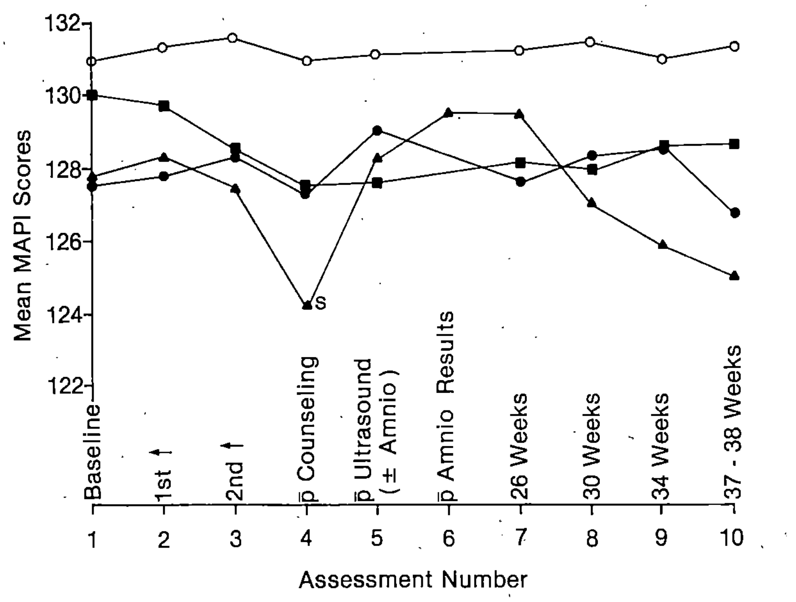

Fig. 3. MAPI scores in experimental and control subjects. o–o, Controls; ●–●, group 1; ▲–▲, group 2; ■–■, group 3; *s*, point at which the plotted value is significantly different from control; $p < 0.01$; *p̄*, after.

trol subjects was observed at only one point in time and for a single subgroup only. Subjects in group 2 exhibited a significantly lower Maternal Attitude to Pregnancy Instrument score, reflecting a more negative attitude toward pregnancy, at the time of the fourth assessment (following counseling but prior to ultrasonography).

No significant difference was found between experimental and control subjects with regard to symptoms of depression, marital harmony, time lost from work, or work stoppage because of pregnancy.

Comment

The considerable data that have been generated by pilot studies of maternal serum α-fetoprotein screening in the United States indicate that this is an effective screening tool that can be successfully integrated into routine obstetric practice. That such screening is beneficial to those patients found to have a fetus with a neural tube defect seems clear. Questions remain regarding the impact of screening on the much larger group of patients who do not have a fetus with a neural tube defect or other detectable anomaly. The effect of screening on patient anxiety has been studied in Sweden by Berne-Fromell et al.[13] who found lower levels of anxiety in mid and late pregnancy in women living in an area with a maternal serum α-fetoprotein screening program as compared to women living in an area without access to screening. In addition, they found that women who accepted screening and had normal results and those who refused screening exhibited similar degrees of anxiety in mid pregnancy whereas those who accepted screening and had initially abnormal results were less anxious.[14] During the period of testing, however, they demonstrated by interview severe emotional distress in those patients with abnormal serum α-fetoprotein results.[15] Fearn et al.[16] studied this issue in the United Kingdom by administering the State-Trait Anxiety Inventory to patients with maternal serum α-fetoprotein elevations during the course of their follow-up assessments and again 2 to 3 weeks later. They found the patients notified of maternal serum α-fetoprotein elevations to be significantly more anxious than normal primigravid women tested several years earlier at their first prenatal visit. In addition, they found that patients who did not require amniocentesis because a benign explanation was found for their maternal serum α-fetoprotein elevations were significantly more anxious than patients who had amniocentesis with normal results 2 to 3 weeks after testing was completed. Unfortunately, there were no control subjects studied simultaneously, and baseline data on anxiety prior to testing were not available for the patients with maternal serum α-fetoprotein elevations.

Our findings indicate that patients notified of maternal serum α-fetoprotein elevations experience significantly heightened anxiety that persists throughout the period of testing until normal results are ultimately obtained. The anxiety that accompanies two sequential elevations was not alleviated by extensive counseling prior to further testing. In our view, this is not at all surprising. Although a normal outcome still occurs in the majority of cases, the patient with a maternal serum α-fetoprotein elevation faces a substantially increased risk of having a fetus with a birth defect or suffering a fetal loss. The fully informed patient is aware of these risks, and counseling alone cannot eliminate the feelings they engender. Although the data indicate that counseling has no immediate impact on anxiety, this does not diminish the need to include counseling in maternal serum α-fetoprotein screening programs. Counseling prior to further definitive testing may well be of benefit to the patient in understanding and coping with subsequent findings, whether normal or abnormal. It is reassuring to note in our study that anxiety among patients with maternal serum α-fetoprotein elevations returned promptly to control levels following notification of normal test results and remained similar to control levels throughout the remainder of the pregnancy. Male partners of patients with maternal serum α-fetoprotein elevations were clearly affected by the experience in that they too exhibit significantly heightened anxiety during the period of follow-up testing.

Our data suggest that there is little variation in maternal attitudes toward pregnancy between 16 and 38 weeks' gestation and that attitudes are relatively unaffected by maternal serum α-fetoprotein elevations. A significantly more negative attitude was demonstrated in group 2 only, and this was observed only at a single point in time—after counseling and prior to ultrasonography. It is difficult to imagine why a difference should be noted between groups 2 and 3 at this time, since the groups had a similar experience up to this point and were only later differentiated by the results of the ultrasound evaluation. Therefore, it would appear that this could be a spurious observation. An alternative explanation for our findings with the Maternal Attitude to Pregnancy Instrument is that this instrument may not be sensitive to temporal fluctuations in attitudes and therefore may not be well suited to repeated administrations. It has been used sequentially in at least one other study[17] but in that case, as in ours, few changes were observed over time.

In summary, we conclude that maternal serum α-fetoprotein screening has a significant psychological impact on patients with maternal serum α-fetoprotein elevations by producing significantly heightened anxiety that persists throughout the sequence of follow-up testing. This occurs even in an optimal setting in which good communication and extensive counseling are provided. In this setting, however, there is no evidence that residual anxiety persists after testing is completed. We do not feel that the anxiety associated with maternal serum α-fetoprotein screening is an argument for eliminating informed consent, nor do we feel that it negates the merits of such screening programs. We do feel, however, that it should not be ignored in weighing the relative risks and benefits of such a program. Most importantly, it underscores the necessity to minimize the time elapsed between patient's initial maternal serum α-fetoprotein elevation and final diagnosis and to minimize the number of false positive results while still maintaining an acceptable detection rate for neural tube defects.

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Myotonic muscular dystrophy associated with ritodrine tocolysis

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A patient thought to be normal was admitted with premature labor at 29+ weeks' gestation. Treatment with the β-mimetic ritodrine hydrochloride appeared to provoke symptoms of myotonic muscular dystrophy. Neurological history and examination confirmed the presence of previously unsuspected myotonic dystrophy in the patient, her father, and paternal grandfather. Discontinuation of the drug led to improvement in myotonia symptoms but worsening premature labor. Magnesium sulfate did not provoke the same symptoms but was unsuccessful in controlling premature contractions. Long-term management with bed rest, phenytoin, and isoxsuprine hydrochloride resulted in term delivery. Subsequently, maternal symptoms of myotonia disappeared. Congenital myotonia was suspected in the fetus because of the ultrasonic demonstration of polyhydramnios and reduced fetal movements. This was confirmed at delivery. The mechanism by which ritodrine unmasked the myotonia is unclear but may be related to changes in the cell membrane (chloride conductance, the sodium-potassium pump, or membrane polarization). (AM J OBSTET GYNECOL 1985;151:83-6.)

Key words: Myotonic muscular dystrophy, ritodrine complications, preterm labor

Myotonic muscular dystrophy is a multisystem disease inherited as an autosomal dominant which in the adult form is recognized by progressive muscle weakness, atrophy (particularly of the face and neck muscles), and myotonia, or the inability to relax the muscles. Other features can include baldness, cataracts, testicular atrophy, and endocrine gland dysfunction. Oftentimes, these symptoms are not recognized in the adult as a disease state, nor is the familial inheritance fully appreciated.

A distinctly different congenital form of the disease was defined in 1960. Harper[1, 2] reported a large series (70 cases) of congenital myotonic dystrophy, reviewing characteristics of the involved pregnancies. In the congenital form of the disease, clinical features are prominent at birth and include facial diplegia, hypotonia, talipes, and respiratory problems. In infancy there is delayed motor development and eventually mental retardation becomes apparent. The pregnancies are often complicated by polyhydramnios, preterm labor, and perhaps dysfunctional uterine contractions. In the case presented here, use of the β-mimetic ritodrine hydrochloride provoked myotonic muscle contractions and led to the diagnosis of myotonic muscular dystrophy.

Case report

On September 15, 1982, at 29+ weeks' gestation, C. S. was admitted to Evanston Hospital, complaining of uterine contractions every 2 to 8 minutes for the past 5 hours. She noted a 2-day history of vague malaise and urinary urgency but denied fever, chills, or gastrointestinal symptoms. Results of physical examination were as follows: temperature, 97.7° F; pulse, 80 bpm; blood pressure, 94/50 mm Hg; respirations, 18/min. She was 5 feet, 1 inch tall, and weighted 105 pounds. Heart, lungs, neck, breasts, and abdomen were normal. Fundal height was 29 cm. Fetal weight was estimated as 1300 gm. Moderate contractions were occurring every 2 to 3 minutes. The abdomen and bladder were nontender. The vagina contained mucoid discharge, no blood, and no amniotic fluid. The cervix was closed, 1 cm long, soft, and posterior, and the fetus was ballotable.

A diagnosis of premature labor and possible urinary tract infection was made, and the patient was admitted for rest and intravenous hydration with 5% dextrose in lactated Ringer's solution. Two hours after admission, contractions were still occurring every 4 to 7 minutes and were subjectively stronger. The cervix was unchanged except for a perceived forward movement from posterior to the midposition. Based on this change and continuing contractions, tocolysis was initiated with intravenous ritodrine hydrochloride (Yutopar) solution, 50 μg/min for 15 minutes, then at 100 μg/min. There was a prompt response to the ritodrine. The patient noticed flushing and palpitations. The pulse rose from 68 to 117 bpm, and blood pressure remained stable at 116/46 mm Hg. The uterine contractions

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stopped after 1 hour at 100 μg/min and the ritodrine was decreased to 50 μg/min. The pulse slowed to 102 bpm, and the patient noticed diminshed flushing and palpitations. Contractions remained arrested.

Nine hours after initiation of the ritodrine, there were still no contractions, and the intravenous ritodrine was changed to oral ritodrine, 10 mg every 2 hours. An ultrasound scan at that time showed a living singleton fetus in a breech position with a biparietal diameter of 7.7 cm (twenty-fifth to seventy-fifth cephalic growth percentile, compared to a previous scan), an estimated fetal weight of 1350 gm, and a greater than average amount of amniotic fluid.

On the afternoon of the following day (hospital day 2), she complained of cramping of the fingers and, when she ate, stated, "It feels like my jaw is cramping or locking." Serum potassium was found to be 2.6 mg/100 ml and she was given 40 mEq of potassium supplement orally twice. Just past midnight (hospital day 3), contractions resumed and intravenous ritodrine was restarted, requiring 150 μg/min to stop the contractions. The patient compained again of the "locking" jaw. Serium calcium was 8.7 mg/100 ml. In the morning, she complained of inability to relax the jaw or the hands without great effort. One gram of calcium gluconate was given intravenously with no change in symptoms. The tentative diagnosis of myotonic muscular dystrophy was made and neurological consultation sought.

Neurological history revealed: (1) For several years, the patient noticed back and leg cramps after jogging, which required massage for relief. (2) For the last 2 years, an aching jaw and stiffness followed long or unusually stressful days at work. (3) There was no recognized family history of muscle problems, but her father (age 56) had frontal baldness and temporal muscle thinning and had developed cataracts in his 40s. Her grandfather lived into his 80s and also had cataracts. Neurological examination at this time showed: (1) percussion myotonia of both thenar eminences, brachioradialis, and tongue, requiring >30 seconds to resolve, and myotonia of hand grip; (2) normally active jaw-jerk reflex; (3) normally active tendon reflexes; (4) normal strength; (5) a slender face with temporal atrophy.

With the presumption that the myotonia symptoms were provoked by potassium shifts to the intracellular space, the ritodrine was discontinued. That evening, the contractions became stronger and more frequent, resulting in a shortening of the cervix to 4 mm in length. To control these contractions, 4 gm of magnesium sulfate was given intravenously in 10 minutes, followed by 1.5 gm/hr and then 2 gm/hr. This regimen reduced the frequency of contractions to every 15 to 20 minutes and the intensity to mild. The myotonic muscle contractions improved markedly and the patient was stable without cervical change for the next 2 days.

On day 6, the contractions were again strong and occurring every 2 to 3 minutes. Serum magnesium was 6.4 mg/100 ml. Reflexes were <1+, and respirations were 8/min. Although no further change in the cervix was noted, the impression was that loss of control of premature labor was occurring, and the magnesium sulfate was stopped. An initial loading dose of intravenous isoxsuprine hydrochloride (Vasodilan) was given, and then a dosage of 12 mg/hr was administered and was effective in stopping the contractions. Phenytoin sodium (Dilantin), 100 mg orally twice a day, was begun to reduce the myotonic symptoms. A trial of oral isoxsuprine failed, and thereafter the patient was maintained on a regimen of intravenous isoxsuprine. Worsening myotonic symptoms necessitated an increase in dosage of phenytoin to 100 mg three times daily. Long-term management consisting of bed rest, weekly nonstress tests, weekly ultrasound examinations, and periodic electrolyte and glucose determinations was planned.

Weekly ultrasound scans demonstrated normal fetal growth, the presence of fluid in the fetal stomach, increased amounts of amniotic fluid, and alternating breech and cephalic positions. By the thirty-second week (hospital day 21), frank polyhydramnios was diagnosed. At the thirty-third week (day 28), cephalic growth was at the ninety-fifth percentile, and at the thirty-fourth week, no fetal breathing could be seen over 30 minutes of observation. Nonstress tests during this time were reactive (normal).

On day 43 (36 weeks), amniocentesis was done. The lecithin/sphingomyelin (L/S) ratio was 2:1 (not fully mature in this laboratory), the creatinine level was 1.0 mg/100 ml, and no phosphatidylglycerol was present. The amniocentesis was repeated 10 days later at 37.5 weeks, and at that time, the L/S ratio was 3:1 (mature) and phosphatidylglycerol was present. The creatinine level was 1.1 mg/100 ml. The following day (fifty-fourth day in the hospital), the isoxsuprine was stopped. She continued to have contractions every 20 minutes as she had done virtually throughout her hospitalization, but no change in the cervix resulted. She was discharged home from the hospital, on a regimen of phenytoin, 100 mg three times daily, with minimal myotonia symptoms.

She was readmitted on December 2, 1982, at term with the fetus in the breech position. Cesarean section was chosen for the delivery method in this nulliparous patient with hydramnios, a floating presenting part, and an unfavorable cervix. The following day, she underwent primary cesarean section through a transverse abdominal Maylard incision and a low cervical transverse uterine incision. Lumbar epidural anesthesia was used, but proved ineffective, so 30 ml of 0.75% Xylocaine hydrochloride was used locally for relief of operative pain. The female infant weighted 7 pounds, 12 ounces and had Apgar scores of 5 and 7 at 1 and 5 minutes, respectively. Thick meconium was present, and despite the immediate use of DeLee trap suctioning, meconium aspiration occurred. The baby was noted to have poor muscle tone and amblyopia and was obviously affected by the more severe congenital form of myotonic dystrophy. The baby suffered transiently from supraventricular tachycardia and poor feeding but subsequently improved and was discharged home 3 weeks after delivery.

The mother did well after operation, her only complaint being muscle weakness, attributed to prolonged bed rest. She continued on a regimen of phenytoin until 6 weeks post partum at which time it was discontinued. Three months after delivery, the mother had no symptoms of myotonic dystrophy and was continuing to regain muscle strength.

Comment

Myotonic muscular dystrophy usually has its onset in early adult life, with death occurring in the fifth or sixth decade. Genetically, the disease has a high penetrance but great variability of expression.[3] Notable features are neck and facial muscle weakness and atrophy resulting in a characteristic expressionless facies with ptosis as the temporalis, sternocleidomastoid, and levator palpebrae muscles are affected. Characteristic cataracts and retinal changes can occur.[4, 5] Associated problems are abnormal glucose tolerance, baldness, gonadal atrophy, and cardiac arrhythmias.[3]

In general terms, the disease results from membrane dysfunction. In the muscle, there is repetitive discharge of the muscle membrane, independent of nerve activity. Use of the β-mimetic agent ritodrine hydrochloride has not previously been reported to provoke the severe myotonia symptoms that occurred in this patient. One of the well-known effects of ritodrine, however, is a shifting of potassium from the extracellular space to the intracellular space. This shift is not usually associated with adverse effects and use of potassium supplements has been considered unnecessary.[6, 7] In this case, a low serum potassium value was obtained, but no short-term improvement in the myotonia was evident following potassium replacement.

At the present time, the membrane defect occurring in myotonic dystrophy is not clearly characterized. Consequently the mechanism of this ritodrine-induced dysfunction cannot be inferred. In goat myotonia and human myotonia congenita, the muscle membranes have been found to have decreased chloride conductance, which can be shown experimentally to result in repetitive action potentials.[8, 9] In adult myotonic dystrophy, however, the membranes seem to have normal resistance and normal chloride and potassium conductance.[3] Investigators have found changes in membrane electrical potential in myotonic dystrophy, but the significance of this is not clear.[10, 11] Additional findings that may ultimately lead to an understanding of the pathophysiology characteristic of myotonic dystrophy derive from red blood cell membrane studies in which reduced phosphorylation of membrane proteins and a change in the stoichiometry of the sodium-potassium membrane transport system have been found.[12, 13] Lastly, phenytoin, an effective therapeutic agent to reduce myotonic symptoms, has been shown to normalize membrane "fluidity" as measured by electron spin resonance in red blood cells from myotonic dystrophy patients.[14] Further confounding and contradicting findings, however, are two reports where use of the β-blocking agent propranolol led to myotonic muscle dysfunction,[15, 16] whereas in our case the β-mimetic agent ritodrine also provoked myotonia in a suceptible patient.

Harper[1, 2] has reported the largest series of this "congenital myotonic dystrophy." Facial diplegia was present in 61 of the 70 infants. Hypotonia (47 of 70), talipes (33 of 70), and respiratory problems (33 of 70) were often present. Delayed motor development of the neonate was present in 60 of the 70, and mental retardation in 48 of the 70. In contrast to the adult form, cataracts were absent in infancy.

The disease features found in the neonate logically have corollary characteristics in utero. Most important, although not understood, is the fact that the congenital form of the disease is almost invariably inherited from the mother. Obstetric problems attributed to maternal disease have included exacerbation of muscle weakness at about 7 months' gestation,[17, 18] premature labor,[18] dysfunctional labor,[17, 19] and failure of maternal voluntary muscle effort. Polyhydramnios occurs frequently when the fetus has the congenital form of myotonia dystrophy, a fact attributed to the affected fetus's reduced ability to swallow amniotic fluid. Delayed swallowing of the contrast medium Hypaque, injected into the amniotic cavity, was demonstrated by Dunn and Dierker.[20] Polyhydramnios was present in 36 of 70 in the Harper series, and reduced fetal movements were also appreciated (24 present, 28 absent, 18 uncertain). Lastly, a high number of fetal and neonatal losses is retrospectively found in mothers with the diagnosis made in subsequent pregnancies because of an affected but surviving infant. Webb et al.[21] reported one family affected with myotonic dystrophy in which an unusual number of prior pregnancies complicated by polyhydramnios, preterm labor, dysfunctional contractions, retained placentas, and pospartum hemorrhage occurred.

A major feature of the adult form of myotonic dystrophy is the variability of symptoms such that, in many instances, affected mothers and families do not recognize the condition as a disease or appreciate the inheritance that is occurring. In Harper's[1] series, 20 of 54 mothers were asymptomatic and 14 had only minor symptoms. In our case, the mother had noted only minor muscle symptoms (aching, stiffness) following extraordinary exertion. The patient's father, from whom she apparently inherited the condition, had frontal baldness, temporal thinning, and cataracts at an early age, and his father had only cataracts.

The anesthetic management of myotonic dystrophy is somewhat complex and deserves comment. General anesthesia should be avoided whenever possible. Since the myotonia originates in the muscle membrane, Pavulon and curare are of no help in terminating the muscle contractures. Neostygmine and other cholinesterase inhibitors used to reverse muscle relaxation cause severe contractures and should be avoided. Depolarizing drugs like succinylcholine lead to severe contracture and should not be given. Thiopental produces severe apnea in these patients and was thought to have caused five deaths in one series.[22] Arrhythmias, including ventricular tachycardia and fibrillation, are seen intraoperatively, especially during general anesthesia. Perioperative shock, if unresponsive to the usual vasopressors, should be treated with steroids. Last, these patients are prone to pulmonary failure and cardiac arrhythmia after operation.[21]

In summary, while ritodrine stabilizes or blocks neuromuscular conduction in the uterus, thus providing a beneficial effect in prolonging pregnancy, it can concurrently upset the membrane balances in other systems with resultant tachycardia, hyperglycemia, hypokalemia, and pulmonary edema.[23] In this case report, ritodrine destabilized muscle membranes of a susceptible individual and resulted in myotonic muscular contractions and a diagnosis of previously unrecognized myotonic cystrophy.

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Pseudomembranous colitis following prophylactic antibiotic use in primary cesarean section

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A report of a hospital outbreak of pseudomembranous colitis in three patients given prophylactic antibiotic therapy before and after primary cesarean section is presented. All patients shared the same ward and labor and delivery room, and the colitis occurred within an 8-day period. The diagnosis of pseudomembranous colitis was suspected clinically and confirmed by limited colonoscopy and biopsy followed by stool culture and toxin assay for *Clostridium difficile.* The high carrier rate of *Clostridium difficile* in the female urogenital tract and altered colonic motility during pregnancy, in addition to antibiotic use, may have contributed to the establishment of this disease. When diarrhea develops postoperatively in patients who have undergone cesarean section, pseudomembranous colitis as a potential serious complication must be kept in mind and necessary precautions taken to impede cross-contamination and development of secondary cases. (AM J OBSTET GYNECOL 1985;151:87-9.)

Key words: Pseudomembranous colitis, cesarean section, antibiotic prophylaxis

Recent reports of clustering pseudomembranous colitis suggest that cross-contamination or environmental factors may be important in the spread of this disease. *Clostridium difficile* has been isolated from the contacts and surroundings of hospitalized patients suffering from pseudomembranous colitis. Also, as pseudomembranous colitis develops more commonly after prolonged antibiotic use, it is rarely described among obstetric patients. In this paper we report a hospital outbreak of pseudomembranous colitis in three women who underwent primary cesarean section after having received perioperative antibiotic prophylaxis.

Case reports

Case No. 1. G. R., a 34-year-old woman, gravida 3, para 2, was admitted on May 12, 1982, at term. After failure of progression of labor because of cephalopelvic disproportion, she was delivered by primary cesarean section. One half hour before delivery, 2 gm of cefoxitin (Mefoxin, Merck, Sharp & Dohme, West Point, Pennsylvania) was administered intravenously. The antibiotic was continued at 6-hour intervals for a total dosage of 19 gm. On May 15, she developed watery diarrhea, diffuse abdominal cramps, and fever to 101° F. Subsequently, the severity of the diarrhea and abdominal cramps intensified, and on May 20 the fever peaked at 103° F, with a white blood cell count of 30,000/mm^3. A diagnosis of pseudomembranous colitis was entertained, and limited colonoscopy to 35 cm was performed (Fig. 1). Endoscopy revealed whitish plaques varying in size from 2 mm to 1 cm with intervening edema, friability, and erythema. Biopsy of these lesions demonstrated partial necrosis of the overlying mucosa with a thick covering of fibrin and inflammatory debris (Fig. 2). Vancomycin (Vancocin, Eli Lilly & Co., Indianapolis, Indiana), 250 mg orally every 6 hours, was started with resolution of symptoms over a 4-day period. Stool culture was positive for *C. difficile.*

Case No. 2. N. A., a 31-year-old woman, gravida 2, para 1, was admitted on April 28, 1982, for possible abruptio placentae. The patient was confined to bed until May 17, when vaginal bleeding occurred, and cesarean section was performed. She received cefoxitin, 2 gm intravenously one half hour preoperatively, followed by four additional doses for a total of 10 gm. On May 20, diarrhea developed and increased in severity over the next 4 days. On May 25, the diagnosis of pseudomembranous colitis was suspected, and limited colonoscopy with biopsies was performed. Classical pseudomembranes were seen in all areas inspected supporting the diagnosis of pseudomembranous colitis. Histologic examination of biopsy specimens was confirmatory. Vancomycin, 250 mg orally every 6 hours, was started and continued for 10 days with prompt resolution of symptoms. *C. difficile* was isolated from cultured stool specimens.

Case No. 3. B. L., a 22-year-old woman, gravida 1, para 0, was admitted in active labor on May 18, 1982.

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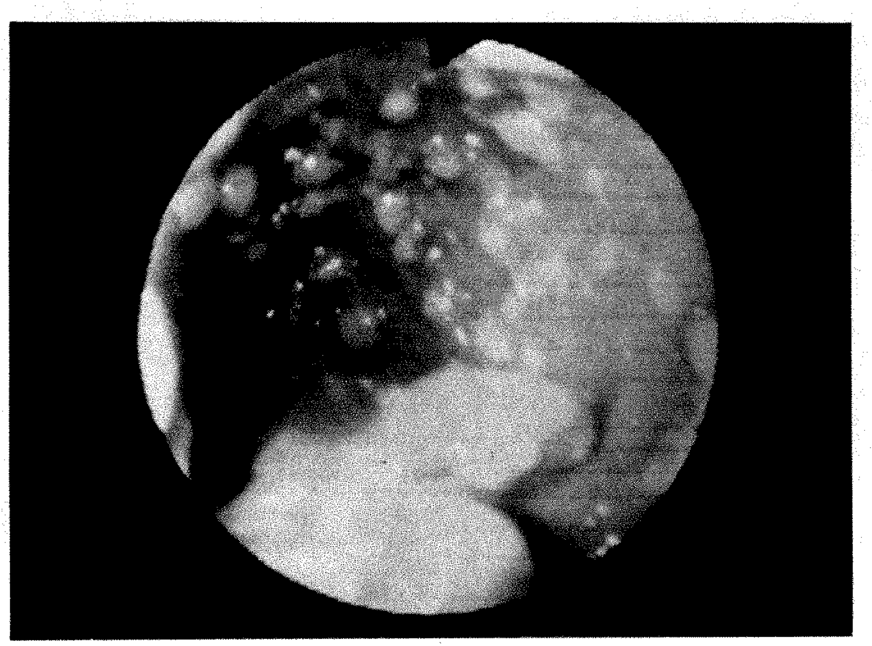

Fig. 1. Endoscopic appearance of classical pseudomembranes with raised white plaques and reddened intervening area. (Original magnification × 10.)

Fetal distress became apparent, with fetal monitoring indicating evidence of cord compression. A cesarean section was performed, and cefoxitin, 2 gm intravenously, was given prophylactically. This was repeated 4 and 8 hours later for a total dose of 6 gm. On May 22, diarrhea, abdominal pains, fever of 101° F, and leukocytosis to 26,000/mm^3 were noted. On May 24, colonoscopy revealed acute colonic inflammation with erythema and exudate in all areas. No classical pseudomembranes were seen, but *C. difficile* culture was positive, and toxin assay was also positive at a titer of 1×10^2. Vancomycin, 250 mg orally every 6 hours, was started with defervescence and decreasing frequency of diarrhea over 2 days.

Comment

In this report we present data supporting cross-contamination as a factor in the spread of pseudomembranous colitis. Although no evidence of *C. difficile* in the environment or on personnel was found during a survey conducted immediately after identification of the third case, the three patients shared in common the labor room and a four-bed ward room. G. R. and N. A. were located in adjacent beds prior to and during the former's onset of diarrhea. With isolation of G. R., Patients N. A. and B. L. eventually occupied the same ward. It seems likely that the spread of the infective organism began with the onset of diarrhea in G. R. on May 15, 1982. The organism was transmitted to N. A. who, in turn, infected B. L. (G. R. and B. L. never having direct contact with each other).

In a 3-month retrospective and 6-month prospective analysis of all women delivered at St. Vincent's Medical Center of Richmond (a total of 1305 deliveries with a cesarean section rate of approximately 25%), no other cases of pseudomembranous colitis were detected. These data, added to the facts regarding the similarity of the circumstances of delivery, and the postdelivery setting lead us to believe that these three cases represent an outbreak of pseudomembranous colitis. It is significant that no patient having a vaginal delivery developed pseudomembranous colitis, yet the role of perioperative antibiotic use is unclear.

Although cefoxitin was given for prophylaxis alone, the suggested protocol of Polk et al.[1] was adhered to only in the case of B. L. The development of diarrhea after a course of prophylaxis with an extremely short incubation period should not dissuade one from entertaining the diagnosis of pseudomembranous colitis, as it has been described with cefazolin,[2] a related antibiotic.

All of our patients received antidiarrheal medication, and two received either ampicillin or a cephalosporin subsequent to the onset of fever and diarrhea, which may have exacerbated the situation.

The lack of identification of *C. difficile* toxin in G. R. and N. A. is bothersome as toxin positivity is present in the vast majority of cases of pseudomembranous colitis. A delay in specimen processing for toxin titers may have played a role. However, all three did have positive stool cultures for *C. difficile*.

The origin of *C. difficile* in these cases is subject to speculation. In addition to the knowledge that there exists a 3% healthy carrier rate, the fact that 40% of neonates harbor *C. difficile* leads us to believe in the

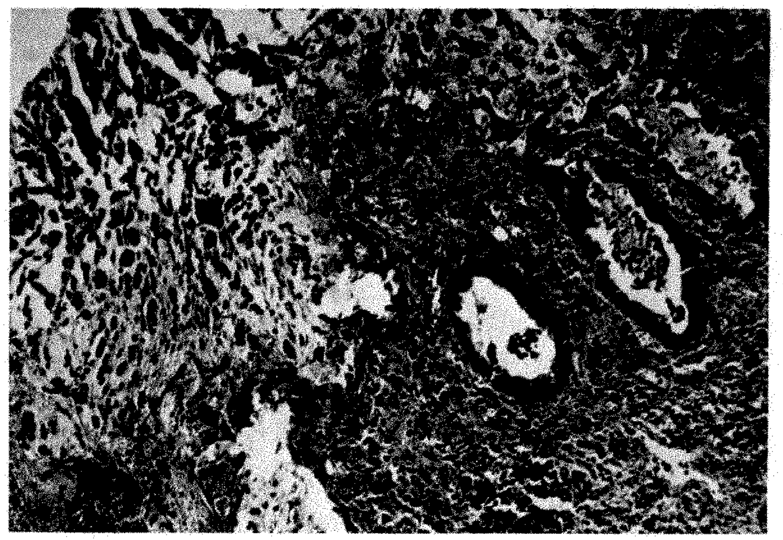

Fig. 2. Photomicrograph of colonic biopsy showing partial necrosis of overlying mucosa and inflammatory exudate. (Original magnification ×400.)

possibility of contamination from the medical and nursing staff that had access to both groups of patients. Altered colonic motility exists in pregnancy as the result of hormonal influences, and this may facilitate growth of *C. difficile* in patients who otherwise would not be susceptible. A high carrier rate in the female urogenital tract may also be contributory.

In conclusion, we wish to stress the following: The occurrence of fever and diarrhea in a postpartum patient who has received antibiotic prophylaxis for cesarean section should bring pseudomembranous colitis to mind, and appropriate measures for rapid diagnosis must be instituted.

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Ultrasonic estimation of weight in the very low–birth weight fetus: A resident versus staff physician comparison

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The estimation of weight in the very low–birth weight fetus (<1500 gm) is becoming more important in obstetric management as neonatal nurseries are reporting better outcome in this weight category. It has become clear that assessment of weight can best be accomplished through the use of ultrasound. In many institutions, however, ultrasonography is under the control of other departments and is not readily available. This arrangement prevents rapid access and compromises the benefit of the technique. To evaluate the accuracy of scans performed in very low–birth weight infants by personnel with limited training in ultrasonography, we undertook a systematic study of weight estimates in this select group of patients. To date, 31 ultrasound examinations have been performed by staff physicians and 50 by resident physicians. Comparisons made between ultrasound examinations by staff and resident physicians showed that the absolute error between the ultrasound-predicted weight and the actual weight, though less in the case of staff physician examinations, was not statistically significant. (AM J OBSTET GYNECOL 1985;151:90-1.)

Key words: Estimation of weight, very low birth weight, ultrasound

The estimation of weight in the very low–birth weight fetus (≤1500 gm) is becoming more important in obstetric management as neonatal nurseries are reporting better outcome in this weight category. It has become clear that prediction of weight in the very low–birth weight fetus can best be accomplished through the use of ultrasound. In many institutions, however, ultrasonography is under the exclusive control of nonobstetric departments and is not readily available to the obstetrician.

In order to evaluate the accuracy of ultrasound scans performed in very low–birth weight fetuses by personnel with limited training in ultrasonography, we undertook a systematic study of weight estimates in this select group of patients.

Material and methods

Eighty-one of the 150 women who were delivered of infants weighing between 500 and 1500 gm from July 1, 1982, to June 30, 1983, at the Women's Hospital (Los Angeles County/University of Southern California Medical Center) had ultrasound scans performed within 7 days of delivery and were eligible for the study. Real-time ultrasonography was performed with the use of a 3.5 MHz transducer. Sonograms were obtained either by obstetric faculty members trained in ultrasonography or by residents in obstetrics and gynecology who worked in the labor and delivery area. The residents in obstetrics and gynecology at the Los

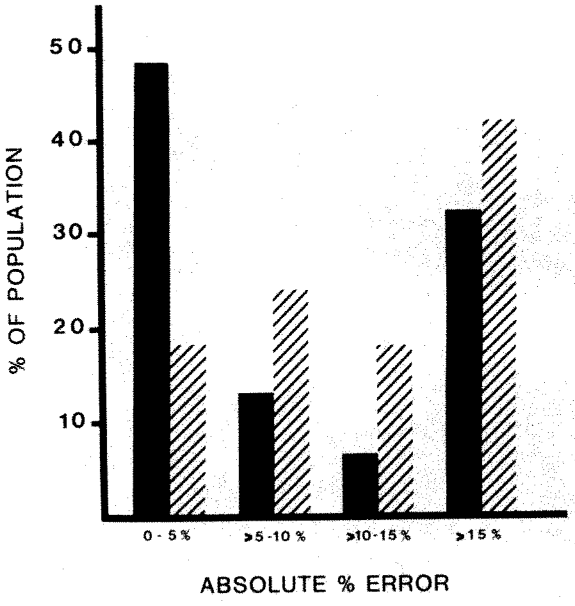

Fig. 1. Fetal weight estimation. This compares the absolute percent error with the percent of patients studied by staff members *(solid bars)* and resident *(hatched bars)* physicians.

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Table I. Error of estimated fetal weight (mean ± SD)

Examination by	*No. of cases*	*Error (gm)*	*Absolute error (gm)*	*Percent error (%)*	*Absolute percent error (%)*	*Error range (gm)*
Staff member	31	−0.45 ±156.29	110.064 ±109.161	0.028 ±13.56	9.99 ±8.98	−420 ±270
Resident	50	−16.60 ±189.47	144.76 ±121.65	−1.70 ±18.20	13.89 ±11.72	−400 ±650

Error = estimated fetal weight − actual birth weight

Absolute error = estimated fetal weight − actual birth weight

$$\% \text{ Error} = \frac{\text{error}}{\text{absolute birth weight}} \times 100$$

$$\text{Absolute } \% \text{ error} = \frac{\text{absolute error}}{\text{actual birth weight}} \times 100$$

Error range = error (maximum) − error (minimum)

Angeles County/University of Southern California Medical Center spend a month training in ultrasonography during the second year of their residency after which they are permitted to use real-time ultrasound in the clinic and labor and delivery area for the duration of their training. Biparietal diameter, abdominal circumference, and femur length measurements were obtained in each fetus.

The charts used to estimate fetal weight for clinical management were based on the formula reported by Shepard et al.,[1] which is

$$\text{Log BW} = -1.7492 + 0.166\ (\text{BPD}) + 0.046\ (\text{AC}) - \frac{2.646(\text{AC} \times \text{BPD})}{1000}$$

where BW = birth weight, BPD = biparietal diameter, and AC = abdominal circumference.

Statistical analysis was performed by means of Student's t test or χ^2, and $p > 0.05$ was considered significant.

Results

Thirty-one ultrasound examinations were performed by the perinatal staff and 50 by residents. A composite error analysis performed on these two groups of patients did not show any significant difference (Table I).

Of the 31 patients scanned by staff members, the estimated fetal weights in 20 patients (64.5%) were within 10% of the actual birth weight, and in 14 patients (45%) the estimated fetal weights were within 5% of the actual birth weight. In the 50 examinations performed by residents, 21 (45%) were within 10% of the actual birth weight and 10 (20%) were within 5% of the actual birth weight (Fig. 1).

Comment

Until the 1960s reports describing outcome in the very low–birth weight infant (≤1500 gm) demonstrated a high mortality rate and prevalence of major handicaps in survivors.[2] With improvement in neonatal care and aggressive obstetric intervention, these ominous statistics have dramatically improved. Controversy exists on how aggressive (cesarean section versus vaginal delivery) obstetric management should be when delivery of the very low–birth weight fetus is imminent.[3] One of the factors that has contributed significantly to clinical management and neonatal survival is the use of ultrasound to predict fetal weight.[4, 5] However, ultrasonography is under the control of departments other than obstetrics and gynecology in most institutions. This arrangement often compromises the immediate availability and diminishes the potential benefit of this technique.

Our results indicate that there was no statistical difference between staff and resident scans (Fig. 1), suggesting that even in the hands of operators with limited experience, ultrasonography can be utilized successfully to improve obstetric care. This modality should be readily available on any labor and delivery floor.

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Evaluation of intra-amniotic administration of 120 gm of urea with 5 mg of prostaglandin $F_{2\alpha}$ for midtrimester termination of pregnancy between 20 and 24 weeks' gestation

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The occasional finding of signs of life in fetuses delivered following intra-amniotic administration of 80 gm of urea and 5 mg of prostaglandin $F_{2\alpha}$ suggested that the dose of 80 gm of urea was inadequate beyond the nineteenth week. Protocol modifications were made, and the metabolic effects of intra-amniotic administration of 120 gm of urea used beyond the nineteenth week were compared to those of the 80 gm dose of urea used in patients prior to the twentieth week. The 120 gm urea dose was well tolerated. The peak blood urea nitrogen (at 4 hours after instillation) was higher with 120 gm of urea (36.4 mg/dl) than with 80 gm of urea (24.6 mg/dl) ($p < 0.05$). Small decreases in the platelet count (14% of control) and serum fibrinogen (11%), sodium (2%), potassium (7%), and carbon dioxide (11%) levels and a 5% increase in peak serum osmolality were found. Following the change in protocol, fetal heart activity has been absent at 3 hours after instillation in all cases <24 weeks from the last menstrual period. (AM J OBSTET GYNECOL 1985;151:92-6.)

Key words: Intra-amniotic urea, intra-amniotic prostaglandin $F_{2\alpha}$, midtrimester abortion

The intra-amniotic instillation of 80 gm of urea with 5 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)[1-3] has been used at the University of Wisconsin for midtrimester termination of pregnancy. In occasional cases, signs of life were present in the fetus at the time of expulsion, suggesting that insufficient concentrations of the intra-amniotic urea had been achieved.[1] This finding occurred mainly in pregnancies >19 menstrual weeks' gestation. A comparative study between urea-$PGF_{2\alpha}$ and saline solution for midtrimester abortion has shown urea to be safer than saline solution and to have a 50% lower incidence of delivery of a live fetus.[3]

Since urea is distributed rapidly across cell walls, its volume of distribution approximates that of total body water. When urea is given intra-amniotically, the concentration is first diluted by any remaining amniotic fluid. There is then an additional drop in urea concentration as the urea is distributed throughout the fetal and placental tissues and a further drop due to the progressive clearance of urea into the maternal compartment.[1, 5]

The amniotic fluid volume in normal pregnancies increases by approximately 3.3-fold between the sixteenth and the twenty-fourth weeks from the last menstrual period, while the combined fetal and placental weights increase by approximately 4.4-fold during this same interval. Since the distribution of urea into the fetal compartment occurs across the fetal skin surfaces, the reduced permeability produced by keratinization of the skin (which begins at 17 weeks and is completed by 25 weeks[4]) combined with the increasing total weight of the fetus and placenta produces progressive decreases in the concentration of urea obtained in both the amniotic fluid and the fetal tissues as pregnancy progresses.

The initial studies on the 80 gm urea dose had included patients from 16 to 20 weeks[6] and from 16 to 22 weeks,[1] leading to a lack of experience beyond 22 weeks and a reduced experience in the 20- to 22-week interval. Since clinical experience elsewhere with a 120 gm dose of intra-amniotic urea had suggested its safety,[7, 8] we increased the dose of intra-amniotic urea to 120 gm for all patients ≥20 weeks' gestation without altering the 5 mg dose of intra-amniotic $PGF_{2\alpha}$. In all cases <24 weeks subsequent to the change in protocol, fetal heart activity has been absent by 3 hours following

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Table I. Comparison of patients given prostaglandin-urea to those given saline solution prior to the protocol modification

	Urea with $PGF_{2\alpha}$	*Saline solution*
Drug-fetus interval (hr), mean ± SD		
< 20 wk	16.9 ± 10.2 (407)*	26.8 ± 10.1 (1263)†
≥ 20 wk	16.6 ± 9.7 (335)†	25.2 ± 7.6 (552)†
All patients	16.8 ± 9.9 (742)	26.3 ± 9.4 (1815)†
Fetus-placenta interval (hr), mean ± SD		
< 20 wk	0.58 ± 0.74 (409)	0.58 ± 1.80 (1275)
≥ 20 wk	0.48 ± 0.61 (334)	0.41 ± 0.61 (554)
All patients	0.54 ± 0.69 (743)	0.53 ± 1.54 (1829)
Hospital days, mean ± SD		
< 20 wk	2.2 ± 0.53 (411)	2.2 ± 0.76 (1204)
≥ 20 wk	2.2 ± 0.49 (327)	2.1 ± 0.44 (501)‡
All patients	2.2 ± 0.51 (738)	2.1 ± 0.68 (1705)†
Hematocrit decrease, mean ± SD		
< 20 wk	1.2 ± 1.5 (395)	1.5 ± 1.8 (1199)‡
≥ 20 wk	1.1 ± 1.5 (316)	1.7 ± 1.9 (529)†
All patients	1.2 ± 1.5 (711)	1.5 ± 1.9 (1728)†
Febrile morbidity		
< 20 wk	3, 0.8% (374)	13, 1.0% (1294)
≥20 wk	5, 1.6% (306)	1, 0.2% (565)§
All patients	8, 1.2% (680)	14, 0.75% (1859)

*Number of patients.

†$p < 0.001$.

‡$p < 0.01$.

§$p < 0.05$.

intra-amniotic instillation of the drugs. We report here data on the metabolic effects of the 120 gm dose in comparison to the 80 gm dose of urea.

Methods

Previous experience at the University of Wisconsin. In the years prior to 1977, 200 ml of 23.4% saline solution was instilled intra-amniotically after removal of variable amounts of amniotic fluid. After 1977, 80 gm of urea in 135 ml of 5% dextrose in water and 5 mg of $PGF_{2\alpha}$ were instilled instead of the saline solution after removal of variable amounts of amniotic fluid. The data from these two intervals were analyzed to compare with results obtained with saline instillation to those obtained with prostaglandin-urea prior to the present protocol modification.

Clinical protocol modifications. The protocol for urea and prostaglandin instillation[1, 2] was modified as follows: (1) As much amniotic fluid as possible was removed prior to instillation of the drugs. (2) Patients <20 weeks from the last menstrual period continued to receive 80 gm of urea dissolved in 135 ml of 5% dextrose in water and 5 mg of $PGF_{2\alpha}$ intra-amniotically. (3) Patients with pregnancies ≥20 weeks and up to 24 weeks from the last menstrual period were given 120 gm of urea in 200 ml of 5% dextrose in water and 5 mg of $PGF_{2\alpha}$ intra-amniotically. (4) The fetal heart was checked with an ultrasound Doppler device at 2 and 3 hours after instillation. (5) Patients with continued fetal heart activity at 3 hours were given a second intra-amniotic instillation as described in items 1 and 3 above. (6) An intravenous infusion containing 100 units per liter of oxytocin was started at 125 ml/hr 4 hours after rupture of the membranes as long as fetal heart movement had ceased to be detectable. (7) The placenta was instrumentally removed if not passed spontaneously by 2 hours following expulsion of the fetus. Venous blood was obtained antecubitally prior to the administration of intra-amniotic drug (time 0) and at 4, 8, 12, and 24 hours following instillation. An additional sample was obtained following placental delivery. We continued to use a 17-gauge, 9-inch Touhy needle for both removal of amniotic fluid and instillation of the urea and then the $PGF_{2\alpha}$. A small amount of the urea solution was saved to flush in the $PGF_{2\alpha}$ dose.

Statistical analysis. Comparisons between groups of different patients were made by t test or one-way analysis of variance, while comparisons between different times within a group of patients were made with the paired *t* test or two-way analysis of variance. Where multiple comparisons to a single control were made, Dunnett's[9] tables for multiple comparisons with a control were used. Log-transformation of the blood urea nitrogen data was necessary because of the wide range of values and to correct for heterogeneity of variance demonstrated with Bartlett's test.[10]

Results

The data for the drug-fetus interval, fetus-placenta interval, hospital days, hematocrit decrease, and febrile

Table II. Drug-fetus interval, fetus-placenta interval, fetal heart, fetal weight, placental weight, and amniotic fluid volume removed in the 80 and the 120 gm of urea groups

	80 gm urea	*120 gm urea*
Drug-fetus interval (hr), mean ± SD	18.4 ± 5.9	14.8 ± 6.3
Fetus-placenta interval (hr), mean ± SD	1.01 ± 0.84	0.61 ± 0.84
Fetal heart at 2 hr (No. detectable)	1	6*
Fetal weight (gm), mean ± SD	197 ± 45	373 ± 88†
Placental weight (gm), mean ± SD	102 ± 36	120 ± 39
Amniotic fluid (ml), mean ± SD	169 ± 66	263 ± 117*

N = 10 for all means except for placental weight in the 80 gm of urea group where N = 9.

*$p < 0.05$.

†$p < 0.01$.

Table III. Clotting factors

	Baseline (mean ± SD)	*After delivery of placenta (mean ± SD)*
Platelets × $10^3/mm^3$ (N = 10)		
80 gm urea	269 ± 32	231 ± 34*
120 gm urea	279 ± 43	252 ± 54†
Prothrombin time (sec) (N = 9)		
80 gm urea	11.44 ± 0.51	12.03 ± 0.54
120 gm urea	11.84 ± 0.58	12.17 ± 0.63
Partial thromoplastin time (sec) (N = 9)		
80 gm urea	25.7 ± 1.9	26.5 ± 1.4
120 gm urea	26.4 ± 1.7	28.0 ± 3.0
Fibrinogen (mg/dl) (N = 9)		
80 gm urea	340 ± 47	301 ± 37‡
120 gm urea	346 ± 64	314 ± 68

There were no significant differences between urea dose groups by one-way analysis of variance. The comparisons within urea dose groups between the baseline and the postplacenta sample were analyzed by paired *t* analysis.

*$p < 0.001$.

†$p < 0.01$.

‡$p < 0.05$.

morbidity for both 5 mg of $PGF_{2\alpha}$–80 gm of urea abortion patients and the earlier saline solution abortion patients, treated at the University of Wisconsin prior to the present protocol change, were compared (Table I). A 9.6-hour decrease in the drug-fetus interval had been produced by the previous switch from the use of saline solution to the use of prostaglandin-urea ($p < 0.001$), a decrease of 37%. A decrease of 0.3 units in the mean hematocrit drop also resulted, a decrease of 20%. No change in overall febrile morbidity was detected, but febrile morbidity had increased by 1.4% in the prostaglandin-urea patients ≥20 weeks ($p < 0.05$). An overall increase of 0.1 hospital day in the urea-$PGF_{2\alpha}$ patients was also detected. This increase was found only in pregnancies ≥20 weeks ($p < 0.01$).

After the recent protocol change, all patients with pregnancies <20 weeks were given 80 gm of urea and 5 mg of $PGF_{2\alpha}$ intra-amniotically while all patients >20 weeks were given 120 gm of urea and 5 mg of $PGF_{2\alpha}$ intra-amniotically. The first 10 patients in each group were monitored intensively to rule out any adverse effect due to the change in protocol. There was no significant difference in the drug-fetus interval or the fetus-placenta interval between the two groups for these 20 patients (Table II). The fetal weights ($p < 0.025$) and number of patients with detectable fetal heart activity ($p < 0.01$) were significantly greater in the pregnancies ≥20 weeks, but the trend toward higher placental weights failed to achieve statistical significance in this small sample. A difference of 94 ml was found in the mean volume of amniotic fluid that could be withdrawn in the two groups ($p < 0.05$).

The values for platelet count, prothrombin time, partial thromboplastin time, and fibrinogen for the baseline and the postplacental samples are shown in Table III. There were no significant differences between the 80 gm and the 120 gm urea dosage groups by one-way analysis of variance. Paired *t* analysis demonstrated significant decreases in the platelet count after placental delivery in comparison to the baseline sample for both the 80 gm of urea ($p < 0.001$) and the 120 gm of urea ($p < 0.005$) groups. However, the decreases were only 14% and 10%, respectively, for the 80 and 120 gm of urea groups, and all individual values remained within normal limits. One patient in each group was eliminated because of missing data for the remaining parameters. Fibrinogen also showed a statistically significant decrease ($p < 0.05$) in the 80 gm of urea group by paired *t* analysis. A similar but smaller decreasing trend in fibrinogen in the 120 gm of urea group failed to reach statistical significance ($t = 2$ with 8 df). Again, the fibrinogen only decreased by 11%, and all individual values remained within normal limits. No statistically significant differences between the baseline and the postplacental samples were demonstrated within the 80 and 120 gm of urea groups by paired *t* analysis for prothrombin time or partial thromboplastin time. Fibrin split products showed an increase from the base-

Table IV. Blood urea nitrogen and serum electrolytes and osmolality before and after instillation of prostaglandin and urea

	Hours after intra-amniotic instillation of drug				
	0	*4*	*8*	*12*	*24*
Blood urea nitrogen					
80 gm urea					
Geometric mean	6.5	24.6*	22.6*	18.8*	9.5*
95% Confidence limits	5.6-7.7	19.9-30.5	17.8-28.7	14.8-24.0	7.6-11.9
120 gm urea					
Geometric mean	6.5	36.4*†	31.7*†	23.0*	10.1*
95% Confidence limits	5.1-8.2	28.0-47.4	22.7-44.3	16.2-32.8	7.4-13.8
Serum sodium (mmol/L, mean ± SD)					
80 gm urea	137.6 ± 2.0	136.4 ± 1.5	135.1 ± 0.8*	134.8 ± 1.5*	135.8 ± 1.9‡
120 gm urea	137.4 ± 1.8	137.0 ± 1.4	135.7 ± 1.4‡	136.2 ± 1.6	137.2 ± 2.7
Serum potassium (mmol/L), mean ± SD					
80 gm urea	3.99 ± 0.37	3.56 ± 0.31*	3.68 ± 0.29	3.61 ± 0.28‡	3.63 ± 0.26‡
120 gm urea	3.84 ± 0.34	3.63 ± 0.23‡	3.57 ± 0.14‡	3.56 ± 0.16‡	3.70 ± 0.23
Serum chloride (mmol/L), mean ± SD					
80 gm urea	103.2 ± 2.2	104.6 ± 1.8	104.8 ± 1.4	105.1 ± 1.6	102.6 ± 3.0
120 gm urea	104.1 ± 1.8	104.9 ± 2.7	105.8 ± 1.9	106.1 ± 1.6	104.1 ± 1.4
Serum carbon dioxide (mmol/L), mean ± SD					
80 gm urea	25.7 ± 0.9	23.2 ± 2.0*	23.0 ± 1.4*	22.4 ± 1.9*	25.4 ± 1.2
120 gm urea	24.8 ± 1.2	23.0 ± 0.7*	22.0 ± 0.9*	22.2 ± 1.2*	24.0 ± 1.9†
Serum osmolality (mosm), mean ± SD					
80 gm urea	274.2 ± 23.7	286.3 ± 4.1	286.9 ± 5.9‡	284.3 ± 4.0	279.7 ± 7.3
120 gm urea	278.9 ± 3.4	292.0 ± 9.0*	293.6 ± 7.2*	286.7 ± 7.0*	280.9 ± 8.3

N = 9 for all means.

*Comparison within urea dose levels between time zero and the time indicated with use of two-way analysis of variance: $p < 0.01$.

†Comparison between urea dose levels within time intervals: $p < 0.05$.

‡Comparison within urea dose levels between time zero and the time indicated with use of two-way analysis of variance: $p < 0.05$.

line to the postplacental sample in seven of 10 samples in both the 80 and 120 gm of urea groups. There was no clinical evidence of a coagulation disorder in any of these patients.

The data on blood urea nitrogen, serum electrolytes, and serum osmolality (Table IV) were analyzed by two-way analysis of variance for comparisons within each urea dosage group. One patient in each group was eliminated prior to data analysis because of missing data. Statistically significant elevations in blood urea nitrogen were demonstrated at 4, 8, 12, and 24 hours after instillation in both the 80 and 120 gm of urea groups. The peak of blood urea nitrogen was reached at 4 hours, and by 24 hours the value had returned to nearly basal levels. The serum sodium level showed slight decreases from the baseline at 8, 12, and 24 hours in the 80 gm of urea group and at 8 hours in the 120 gm of urea group. Serum potassium also showed slight decreases at 4, 12, and 24 hours in the 80 gm of urea group and at 4, 8, and 12 hours in the 120 gm of urea group. There were no statistically detectable alterations in the serum chloride level. The serum carbon dioxide level showed slight declines at 4, 8, and 12 hours in both groups, and serum osmolality was demonstrated to increase slightly at 8 hours in the 80 gm of urea group and at 4, 8, and 12 hours in the 120 gm of urea group. The mean decreases in serum sodium, potassium, and carbon dioxide levels and the mean increase in serum osmolality were 2%, 7%, 11%, and 5%, respectively. One-way analysis of variance was used for comparisons between dosage groups. The blood urea nitrogen reached significantly higher values in the 120 gm group than in the 80 gm of urea group at 4 and 8 hours, demonstrating increases of 48% at 4 hours and 40% at 8 hours. A statistically significant but numerically small (5%) decrease in carbon dioxide level in the 120 gm of urea group compared to the 80 gm of urea group was demonstrated at 24 hours. There were no other statistically significant differences between groups at any time.

Comment

Our findings of a decreased drug-fetus interval, smaller hematocrit decrease, and increased febrile morbidity in urea-$PGF_{2\alpha}$ patients in comparison to saline solution abortion patients are similar to those of Binkin et al.[3] In our study, the increase in febrile morbidity appeared to be found only in patients ≥20 weeks from the last menstrual period. An increase of 0.1 hospital day in urea-$PGF_{2\alpha}$ patients in comparison to saline solution patients was also found only in patients with pregnancies ≥20 weeks from the last menstrual period. It is not clear whether these factors may be another result of urea underdosing in the group with advanced gestational age.

Previous studies have demonstrated that an intra-amniotic injection of 80 gm of urea produces a peak serum urea nitrogen concentration of approximately 30 mg/dl at 4 hours.[1] Our study has found a peak blood urea nitrogen value of 24.6 mg/dl after an 80 gm of urea dose and a peak blood urea nitrogen value of 36.4 mg/dl after a 120 gm of urea dose, indicating that the blood urea nitrogen peak is approximately directly proportional to the dose of urea administered. In sickle cell patients, 2 gm of urea per kilogram of body weight has been administered intravenously over a 3-hour period, and blood urea nitrogen concentrations up to 200 mg/dl were reported without serious side effects.[11, 12] At the University of Wisconsin up to 1 to 1.5 gm of urea per kilogram is currently used intravenously for management of cerebral edema or during operation for intracranial mass lesions.[13] As much as 4.4 gm/kg has been administered, resulting in massive diuresis.[13] In the maternal compartment, urea acts as an osmotic diuretic, producing rapid clearance of the urea. When high concentrations of urea are achieved in the peripheral blood, intravenous administration of fluid and electrolytes is necessary to prevent dehydration.[11-13]

In the monitored patients reported on here, only slight changes in serum osmolality and electrolytes were noted and were consistent with the expected osmotic effect of the urea and the effect of the urea as an osmotic diuretic. The occasional occurrence of a clinically important coagulopathy in patients undergoing termination of pregnancy with prostaglandin-urea or saline solution is well known.[14] The decreases in platelets and fibrinogen demonstrated by paired *t* analysis in the 80 gm of urea group also were seen but were less prominent in the 120 gm of urea group, and there was no change in the percentage of patients demonstrating an increase in fibrin split products. No cases of clinically significant coagulopathy have occurred since the change in protocol. Despite the 120 gm of urea dose, detectable fetal heart activity persisted longer in the group with a gestational age of ≥20 weeks. More than 150 patients have been treated under the new protocol with the majority >20 weeks. No fetal heart activity was detectable by 3 hours in any patient with a pregnancy <24 weeks from the last menstrual period in either group subsequent to the protocol change. The half-life of the intra-amniotic urea is approximately 3 hours.[1] If fetal heart activity is found at 3 hours, our protocol calls for a second intra-amniotic injection of 120 gm of urea with 5 mg of $PGF_{2\alpha}$ following removal of as much amniotic fluid as possible. The second dose of urea is expected to produce higher concentrations of intra-amniotic urea than were produced by the first instillation since the residual amniotic fluid should still contain a higher concentration of urea than was originally present.

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Fetal pulmonary cyst: Intrauterine diagnosis and management

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This case report describes the antenatal diagnosis of a fetal pulmonary anomaly. Early detection afforded detailed parental counseling and prompt neonatal diagnosis and treatment. (AM J OBSTET GYNECOL 1985; 151:97-8.)

Key words: Fetal pulmonary cyst, ultrasound, diaphragmatic hernia

We report a case illustrating how prenatal ultrasonic diagnosis of a major anomaly influenced patient management to bring about a favorable outcome.

Case presentation

The patient, a 38-year-old primigravid woman, had a normal past medical history and initial physical examination at 12 weeks' gestation. Because of advanced maternal age, an amniocentesis was recommended and performed at the sixteenth week of pregnancy. Prior to the amniocentesis, a standard stage I ultrasound study was carried out to date the pregnancy, rule out twins, establish fetal life, localize the placenta, and define the most suitable location on the maternal abdomen for amniocentesis. The results of amniotic fluid analysis for fetal karyotype and level of α-fetoprotein were normal.

A second ultrasound examination was performed at 23 weeks' gestation to evaluate fetal growth. Although the fetal head and trunk measurements were normal, it was noted that the heart and mediastinum were displaced to the right side of the chest (Fig. 1). The stomach bubble could not be identified in the upper abdomen. Thus, the primary diagnosis was diaphragmatic hernia; the differential diagnosis included pulmonary cyst and adenomatoid cystic malformation of the lung.

The patient and her husband were informed about the presence of a major anomaly in the fetal chest and the differential diagnosis of the abnormality. They were advised that all conditions could be associated with hypoplastic lungs and also that if the defect was, in fact, a diaphragmatic hernia the prognosis was quite grave. The couple elected to continue the pregnancy and expressed their desire to have treatment of the infant be as aggressive as necessary, including cesarean section for fetal distress if indicated.

The remainder of the pregnancy was complicated by the development of gestational diabetes, which was well controlled by diet. Ultrasound examination at 32 weeks showed no change in the abnormal findings. The stomach bubble was still not visualized. Weekly nonstress tests begun at 36 weeks were reactive.

To facilitate attendance of a team of specialists for optimal neonatal care, labor was induced with Pitocin at 39+ weeks of pregnancy, as defined by early ultrasonic parameters. Labor progressed normally, and a male infant weighing 7 pounds, 1 ounce was delivered by low forceps over a mediolateral episiotomy. Pudendal anesthesia was used.

The baby did not breathe spontaneously and was intubated immediately. Since it was known that the infant could be expected to have hypoplastic lungs, excessive pressure with the Ambou bag was avoided so as to prevent pneumothorax. No breath sounds were detected over the left aspect of the chest, and heart sounds were audible adjacent to the right midclavicular line. Chest and abdominal roentgenograms showed multiple air-filled pockets in the left lung area, a right mediastinal shift, and gas within a normally rotated colon within the abdomen (Fig. 2). With these findings, the presumptive diagnosis was a pulmonary cystic lesion.

The baby was transferred to Children's Memorial Hospital and was taken directly to a previously prepared operating room. With the infant under general anesthesia, the pleural space was entered and a large, cystic, left upper lobe was seen along with a small but normal-appearing left lower lobe. A left upper lobectomy was performed. The pathologic diagnosis was cystic adenomatoid malformation of the lung. After operation, the baby did well. At 1 year of age, height was at the seventy-fifth percentile and weight at the fiftieth percentile.

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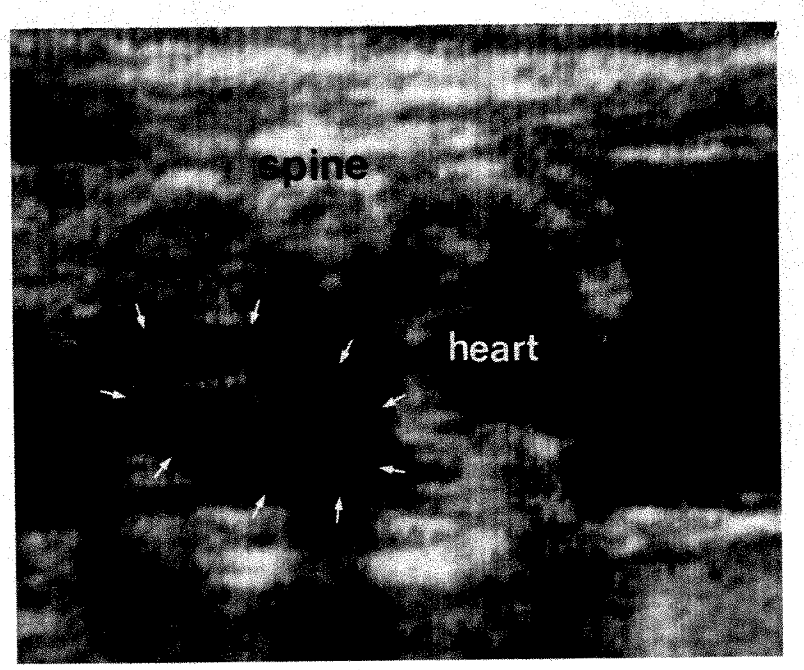

Fig. 1. Echogram showing cross section of fetal chest. Heart is markedly displaced to the right side by a large mass *(arrows)* occupying the left aspect of the chest.

Comment

This case history demonstrates not only the feasibility of antenatal diagnosis of remediable fetal anomalies by ultrasound but also the importance of detailed counseling and individualized management. The differential diagnosis of the chest mass was presented to the parents, who understood that the prognosis might be quite unfavorable. However, they were able to make an informed decision based on available information and were emotionally prepared for the ensuing course of events. Furthermore, the timing of delivery was well controlled and expedited the attendance of a team of specialists to resuscitate the infant, prevent pneumothorax, execute a planned battery of tests, and perform immediate operation on the baby.

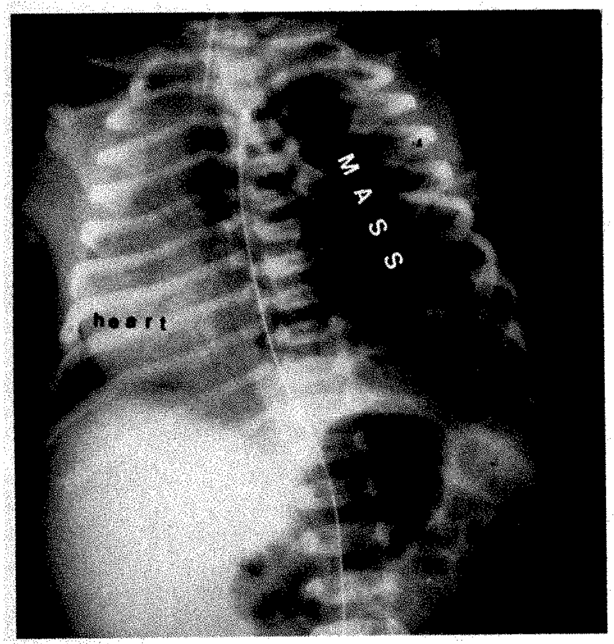

Fig. 2. Chest x-ray film of newborn infant.

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Current Development

Calcium metabolism in pregnancy and the perinatal period: A review

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Calcium homeostasis is a complex process involving calcium, other involved ions, and three calcitropic hormones, parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D_3. The principal maternal adjustment during pregnancy is an increasing parathyroid hormone secretion which maintains the serum calcium concentration in the face of a falling albumin level, an expanding extracellular fluid volume, an increasing renal excretion, and placental calcium transfer. The placenta transports calcium ions actively, making the fetus hypercalcemic relative to its mother, which in turn stimulates calcitonin release and perhaps suppresses parathyroid hormone secretion by the fetus. A unique extrarenal system for 1α-hydroxylation of 25-hydroxyvitamin D_3 exists in the placenta and/or decidua, providing a source of 1,25-dihydroxyvitamin D_3 for the fetus. With the abrupt cessation of the placental source of calcium at birth, the neonate's serum calcium level falls for 24 to 48 hours, then stabilizes and rises slightly. Hyperparathyroidism during pregnancy causes complications in both mother and infant and should usually be treated surgically as soon as diagnosed. Maternal hypoparathyroidism can be treated satisfactorily with high doses of supplemental calcium and vitamin D. Osteopenia accompanying long-term heparin administration may respond to 1,25-dihydroxyvitamin D_3 (calcitriol) therapy. Diabetes in pregnancy is associated with disturbed neonatal calcium homeostasis, perhaps due to chronic hypomagnesemia. A possible etiologic role of calcium deficiency in pregnancy-related hypertension has been suggested. Dietary deficiency of calcium and/or vitamin D during gestation may lead to several adverse effects in the newborn infant. (AM J OBSTET GYNECOL 1985;151:99-109.)

Key words: Pregnancy, calcium, metabolism

Calcium homeostasis consists in essence of the relationship between a large pool of calcium in the skeleton and a much smaller pool in the extracellular fluid, modified by input from the gastrointestinal tract and loss mainly from the kidney. The relationship is an extraordinarily dynamic one with exchange regulated quite precisely so as to maintain the serum ionic calcium level within narrow physiologic limits. The mechanism of calcium homeostasis is multifactorial, involving calcium itself, other related minerals such as magnesium and phosphorus, and three calcitropic hormones—parathyroid hormone, calcitonin, and the active form of vitamin D, 1,25-dihydroxyvitamin D_3.

Calcium metabolism in the pregnant woman, fetus, and newborn infant was the subject of a review published in this JOURNAL in 1975.[1] That review identified many unanswered questions relating to both physiologic and clinical aspects of the topic. Research during the ensuing decade has filled in a number of the gaps in our understanding, particularly in maternal and fetal physiology, and has raised new implications with respect to clinical correlations. It therefore seems appropriate to again review the subject. The present consideration will focus on data accumulated over the past decade, with specific literature citations limited to those published since the earlier review.

Maternal physiology

During the course of gestation, the maternal organism undergoes a remarkable series of physiologic adjustments aimed at preserving maternal homeostasis while at the same time providing for growth and development of the fetus and placenta. Many of these adjustments have direct implications with respect to calcium metabolism—extracellular fluid volume expands, renal function increases, and calcium is transported to the fetus. The net increment in calcium

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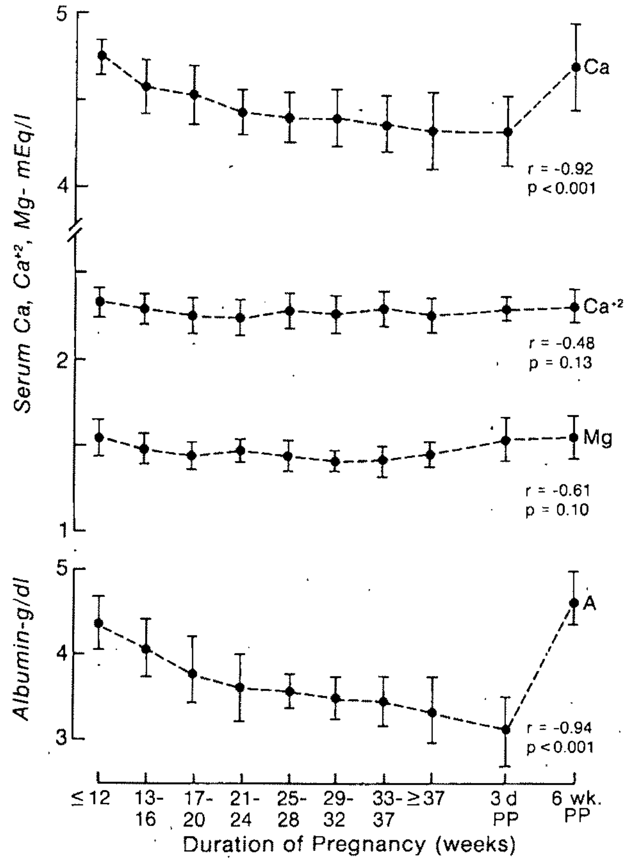

Fig. 1. Mean (±SD) serum levels of total and ionic calcium, magnesium, and albumin in 30 patients studied longitudinally during pregnancy and the puerperium. (Reproduced with permission from Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Calcium metabolism during pregnancy: a longitudinal study. AM J OBSTET GYNECOL 1979;133:781-7.)

accumulation totals 25 to 30 gm by term, most of it represented by mineralization of the fetal skeleton.

Calcium absorption, excretion, and storage. Alterations in maternal calcium metabolism begin shortly after conception, apparently in response to gestational hormones. Intestinal absorption of the mineral is enhanced and the rate of bone turnover increases progressively. Women in the third trimester have consistently positive calcium balances with some correlation between degree of positivity and intake levels (that is, + 142 mg/day with 1 gm intake and + 305 gm/day with 2 gm intake).[2] Enhanced calcium absorption during pregnancy in part reflects high levels of intake but the efficiency of absorption undoubtedly improves as well. The mechanism of calcium absorption involves binding of calcium to a specific protein (calcium-binding protein) whose synthesis is stimulated by active forms of vitamin D. Thus the increased levels of 1,25-dihydroxyvitamin D_3 associated with pregnancy (discussed in detail below) lead to increased levels of intestinal calcium-binding protein and to augmented calcium absorption.

Urinary calcium excretion has been reported in different studies both to increase and to decrease during late gestation. A recent large cross-sectional study[3] found a range of 30 to 620 mg/day, with a distribution skewed toward high excretion (that is, 20% of third-trimester gravid women exceeded 350 mg/day, the 95% value for nonpregnant women). Moreover, calcium excretion correlated significantly with creatinine clearance, implying that the gestational increase in glomerular filtration rate is responsible.

Theoretically, the effect of pregnancy on the maternal skeletal calcium content could be either positive or negative. Some degree of increased deposition has been demonstrated in certain lower animals (but not in others), a situation which would offer certain advantages in storing calcium against the greater needs to come during lactation. On the other hand, it could be argued that many pregnant women ingest amounts less than those needed and therefore must subsidize the difference by skeletal resorption. The issue has been investigated by means of photon absorptiometry or x-ray spectrophotometry but the results appear contradictory. Parous women were found to be somewhat more mineralized than nulligravid women,[4] suggesting increased storage with gestation. One longitudinal study[5] involving measurement of bone density of the distal forearm noted no changes during gestation whereas another[6] found some loss in trabecular bone but none in cortical bone. Thus, while the picture is not entirely clear, it seems likely that pregnancy per se exerts little influence on bone mineral content, at least under normal conditions. However, biochemical evidence of osteomalacia has been found in gravid women in whom low calcium intake is combined with the cultural practice of excluding sunlight exposure from the skin.[7-9]

Serum mineral levels. The total calcium concentration in maternal serum characteristically declines during gestation, reaching a nadir during the middle third trimester and rising slightly thereafter.[10-13] The decline averages about 0.25 mEq/L or 5% to 6% from the preconceptional level, and the pattern parallels that of serum albumin, suggesting strongly that the fall involves the protein-bound fraction. These relationships are illustrated in Fig. 1. Magnesium and phosphorus levels also tend to decline during gestation.

The effect of gestation on maternal serum ionic calcium levels is a question of some considerable physiologic significance with respect to the issue of whether the "physiologic hyperparathyroidism of pregnancy" is primary or secondary. Direct measurement of ionic calcium levels by calcium flow-through electrode has yielded somewhat contradictory results with most reports describing a tendency to a fall[11-15] but one recent study noting a statistically significant rise.[16] All reported changes, however, are small in extent, and it therefore appears that any gestational effect on the serum ionic calcium level is minimal.

Parathyroid hormone and calcitonin. Theoretically, increased parathyroid hormone secretion would represent a logical maternal adjustment to the expanding extracellular fluid volume and calcium loss via urinary excretion and placental transfer. Such does seem to be the case, for nearly all reported investigations[12-14, 17, 18] found parathyroid hormone levels to be elevated at least at some point, usually during late pregnancy. The finding is not quite unanimous,[19, 20] but the majority opinion clearly is that pregnancy represents a state of increased parathyroid activity. The pattern and extent of the gestational increase in parathyroid hormone secretion are less clear. Some studies[12, 17] have suggested that the rise is limited largely to the third trimester and amounts to a doubling of the nonpregnant value whereas others[13] have described a more gradual and progressive increase amounting to 30% to 50% by term. Fig. 2 illustrates the latter type of results obtained in a longitudinal study.

In theory, some increase in calcitonin secretion during pregnancy would seem reasonable physiologically in protecting the maternal skeleton from excessive parathyroid hormone—induced resorption while permitting the latter hormone's gut and kidney effects to continue. Experimental support for this concept comes from findings of decreased bone density with gestation in thyroidectomized rats.[21] Human studies, however, are contradictory with no significant change reported in one,[13] a consistent but usually unchanging elevation in others,[14, 22] and a progressive increase in still others.[19, 23] Fig. 2 contains data from a longitudinal study in which no significant change was found.

Studies of calcitropic hormones during pregnancy have generally been limited to measurement in the basal or unstimulated state. However, responsiveness to provocative stimuli has been described in one investigation[24] involving rhesus monkeys tested at three points during gestation and again post partum. Basal parathyroid hormone levels were found to be elevated but, somewhat surprisingly, the parathyroid hormone response to ethylenediaminetetra-acetic acid–induced hypocalcemia was *less* in pregnancy than in the nonpregnant state and tended to *diminish* with advancing gestation. Calcitonin responsiveness, by contrast, was greater in pregnancy than post partum and tended to increase with time. These findings were thought to reflect different storage characteristics of the two calcitropic hormones, with the net effect that of protecting the maternal skeleton from excessive resorption at times of hypocalcemia and promoting skeletal calcium storage when hypercalcemia occurs.

Vitamin D. Vitamin D_3, either ingested in a preformed state or synthesized in the skin by the action of ultraviolet light, is normally hydroxylated in the 25-position in the liver to form 25-hydroxyvitamin D_3, the principal circulating form of the vitamin. Pregnancy per se exerts little influence on serum 25-hydroxyvitamin D_3 levels[18, 19, 23-28]; a slight decline in late gestation has been reported in some studies,[25, 26] but the major determinant clearly is the amount of exposure to ultraviolet light.[27, 28] Average values of 15 to 20 ng/ml in winter months double or triple during the summer.[27]

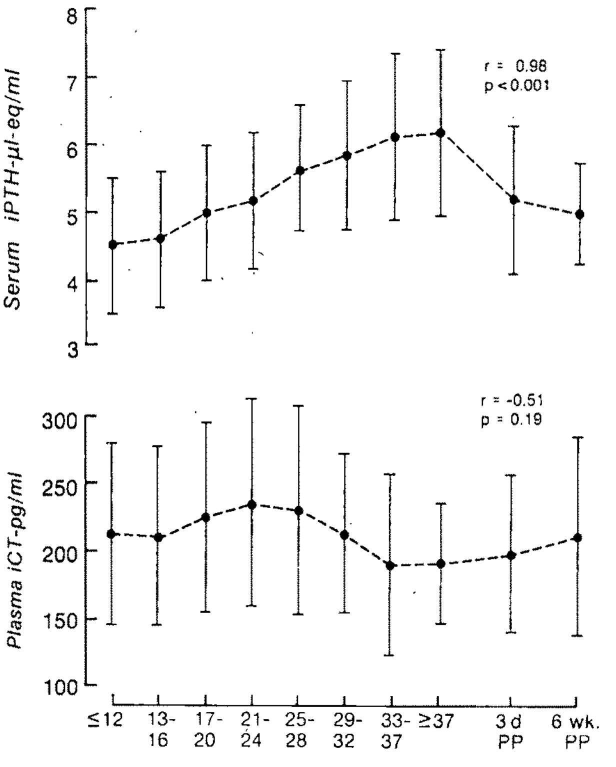

Fig. 2. Mean (±SD) levels of parathyroid hormone and calcitonin in 30 patients studied longitudinally during pregnancy and the puerperium. (Reproduced with permission from Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Calcium metabolism during pregnancy: a longitudinal study. AM J OBSTET GYNECOL 1979;133:781-7.)

25-Hydroxyvitamin D_3 is normally hydroxylated again in the 1α-position in the kidney to form 1,25-dihydroxyvitamin D_3, the biologically active form of the vitamin. 1,25-Dihydroxyvitamin D_3 levels increase substantially during pregnancy, with nonpregnant values approximately doubling to the range of 75 to 100 pg/ml.[19, 29-32] The principal physiologic mechanism promoting renal hydroxylation of 25-hydroxyvitamin D_3 is parathyroid hormone and it is tempting to point to "physiologic hyperparathyroidism" as the cause of increased 1,25-dihydroxyvitamin D_3 formation in the pregnant woman. Other hormones known to promote 1,25-dihydroxyvitamin D_3 synthesis which could be responsible are prolactin, growth hormone, and estro-

Table I. Mineral levels in maternal and cord serum*

Mineral	*Maternal venous* *(n = 115)*	*Umbilical venous* *(n = 115)*	*Umbilical arterial* *(n = 79)*
Ionic calcium (mEq/L)	2.23 ± 0.12	2.82 ± 0.18†	2.81 ± 0.17†
Calcium (mEq/L)	4.25 ± 0.29	5.30 ± 0.37†	5.19 ± 0.27†
Magnesium (mEq/L)	1.31 ± 0.38	1.41 ± 0.38	1.39 ± 0.13
Phosphorus (mg/dl)	4.37 ± 1.61	5.96 ± 1.26†	5.83 ± 1.24†
Albumin (gm/dl)	3.46 ± 0.51	4.16 ± 0.83†	4.04 ± 0.75†

*Data from Schauberger CW, Pitkin RM. Maternal-perinatal calcium relationships. Obstet Gynecol 1979;53:74.
†Values statistically significantly different from maternal venous levels ($p < 0.05$ or better).

gen.[32] However, no significant statistical correlation was found between any of these hormones and 1,25-dihydroxyvitamin D_3 levels in one study,[31] so the mechanism must be regarded as uncertain.

Vitamin D forms are carried in the serum by a specific globulin known as D-binding protein. Levels of D-binding protein characteristically increase during pregnancy so it is not entirely clear that elevation of 1,25-dihydroxyvitamin D_3 means additional free (that is, biologically active) hormone. Bouillon et al.[30] calculated molar ratios to demonstrate that the free level was unchanged until the last month or two of gestation when it increased.

Perinatal physiology

All studies of perinatal physiology suffer from the generic limitation imposed by the relative inaccessibility of the human fetus. Information is available from three potential sources: (1) measurements made on amniotic fluid aspirated from human pregnancies (assuming the amniotic sac to represent an extension of the fetal extracellar space), (2) data from cord blood obtained immediately after birth (which are then extrapolated back to fetal life), and (3) studies in other species whose fetoplacental relationships permit measurements to be made in a relatively undisturbed state. All such data are necessarily indirect and require certain assumptions in applying them to the situation in the human. Nevertheless, certain inferential conclusions with respect to fetal calcium homeostasis can be drawn from such studies.

Placental transfer. It has been appreciated for many years that the serum calcium concentration in the infant at birth (and therefore presumably in late fetal life) regularly exceeds that of the mother, suggesting strongly that the mechanism of placental transfer is one of active transport from mother to fetus. Additional evidence of an active placental transfer system has been provided by kinetic analysis,[33] demonstration of an adenosine triphosphate–dependent system,[34] and calcium localization within endoplasmic reticulum and mitochondria of placental cells.[35] A specific calcium-binding protein, similar to or perhaps identical with intestinal calmodulin, has been isolated from rat[36, 37] and human[38] placentas.

In contrast to calcium, the calcitropic hormones parathyroid hormone and calcitonin do not appear to cross the placenta. Direct studies involving maternal parathyroid hormone administration in the monkey[39] and sheep[40] have indicated a lack of fetal transfer. Although indirect suggestions of calcitonin transfer have been noted in the rat,[41] most reports have indicated an impermeability to this hormone as well.

The evidence relating to placental permeability of the various forms of vitamin D is much more problematic. Direct studies in the sheep indicate some degree of transfer of both 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 and suggest further that the active D vitamin may regulate placental calcium transport.[42] Perfusion studies of the human placenta in vitro also indicate some degree of transfer (greater for 1,25-dihydroxyvitamin D_3 than for 25-hydroxyvitamin D_3) which depends principally on presence or absence of D-binding protein.[43] On the other hand, comparison of maternal-cord values at birth (summarized in detail below) makes it doubtful that the human placenta transports 1,25-dihydroxyvitamin D_3 in vivo.

Fetal mineral levels. Active placental transport of calcium ions from mother to fetus against a concentration gradient, making the fetus hypercalcemic relative to its mother, represents the central feature of intrauterine calcium metabolism. The levels of total and ionic calcium in cord blood regularly exceed those in maternal blood, by an average of 1 and 0.5 mEq/L, respectively.[44-47] Magnesium is marginally higher, and phosphorus substantially so, in cord blood as compared with maternal blood.[44-46, 48] Representative values for these minerals are listed in Table I.

Fetal parathyroid hormone and calcitonin. Assuming that the fetus responds endocrinologically in a manner similar to that of the adult, chronic fetal hypercalcemia during late intrauterine life would be expected to suppress parathyroid hormone secretion and/or stimulate calcitonin release. Although the fetal parathyroid glands contain identifiable hormone from 10 weeks onward,[49] the status of parathyroid hormone responsiveness during fetal life is problematic. On the one hand, amniotic fluid parathyroid hormone levels were noted to decline during late gestation[50] and hormone levels in cord blood significantly below maternal

Table II. Maternal-cord 1,25-dihydroxyvitamin D_3 levels

	1,25-Dihydroxyvitamin D_3 (pg/ml)			
	Maternal	*Cord*	*Correlation (r)*	*Fetal/maternal ratio*
Bouillon et al.[30]	97	48	$p < 0.001$	0.49
Wieland et al.[45]	105*	50*	$p < 0.001$	0.48
Delvin et al.[46]	97	18	NS	0.58
Gertner et al.[69]	77	58	$p < 0.001$	0.75
Fleischman et al.[70]	61	25	NS	0.41
Steichen et al.[71]	63	19	NS	0.30

*Read from graph.

values have been demonstrated in four studies.[45, 51-53] On the other hand, four other reports have found cord levels similar to either maternal or nonpregnant adult values.[46, 54-56] Furthermore, experimental induction of hypocalcemia in the monkey fetus has yielded conflicting results, with lack of response found in one study[57] and prompt parathyroid hormone release in another.[58]

An apparent failure of parathyroid hormone secretion to be suppressed by the relative hypercalcemia of late intrauterine life, noted in some studies of the question, seems paradoxical. One potential explanation involves the possibility of a nonsuppressible source of the hormone in utero. In this regard, it has been noted that parathyroid hormone levels in umbilical venous blood (that is, coming from the placenta), significantly exceed those in umbilical arterial blood (that is, from the fetus), suggesting a placental source of the hormone.[56] Another possibility is that the negative feedback system governing parathyroid hormone release may involve a different "set point" in the fetus exposed to chronic hypercalcemia from that operative after birth. Finally, it may be that these discrepancies reflect the well-known difficulties in parathyroid hormone radioimmunoassay arising from variations in immunoreactivity with the various circulating forms of the hormone.

The fetal status with respect to calcitonin is substantially clearer. The fetal thyroid contains immunologically identifiable calcitonin throughout most of intrauterine life,[59] and acute studies in rhesus monkeys have demonstrated hormone release in response to the provocative stimuli calcium and glucagon.[60] Moreover, reported calcitonin measurements in cord blood significantly exceed maternal or adult values in all reports[23, 45, 51, 52, 56, 61-63] except one.[55] One study[47] found umbilical venous levels to significantly exceed umbilical arterial values (with the difference limited to female infants), leading to the suggestion of the possibility of a placental source of the hormone. In the rat, placental calcitonin levels were higher than serum values and decreased over the last half of gestation,[64] findings consistent with placental elaboration.

Vitamin D. Three forms of Vitamin D are of interest during fetal life—25-hydroxyvitamin D_3 (the principal circulating form), 24,25-dihydroxyvitamin D_3 (a relatively inactive metabolite), and 1,25-dihydroxyvitamin D_3 (the most active biologic form). All have been identified in amniotic fluid in levels 5% to 10% of human serum values.[65]

Cord blood levels of 25-hydroxyvitamin D_3 have been reported in a number of studies which consistently indicate values 20% to 30% lower than concomitant maternal measurements and significantly correlated with them.[45, 46, 51, 52, 55, 66-68] Such a relationship, coupled with findings from direct placental transfer studies summarized previously, implies that transfer of this metabolite from mother to fetus occurs relatively freely.

Placental transfer of 1,25-dihydroxyvitamin D_3 appears to be another matter. From the six publications listed in Table II,[30, 45, 46, 69-71] it can be seen that cord levels amounted to only about half of maternal values and, moreover, statistically significant correlations between the two circulations were found in only three of the six reported studies. Thus, any free transfer of 1,25-dihydroxyvitamin D_3 by the placenta seems questionable.

Several recent studies have documented a capacity for 1α-hydroxylation of 25-hydroxyvitamin D_3 in the nephrectomized pregnant rat.[72, 73] These observations are particularly interesting because in the adult animal the kidney is the only site of 1α-hydroxylation of 25-hydroxyvitamin D_3. At least in the sheep, the fetal kidney lacks 1α-hydroxylase activity,[74] suggesting that extrarenal sources provide all of the fetal supply of 1,25-dihydroxyvitamin D_3. A capacity for 1α-hydroxylation has been documented in in vitro studies of the rat placenta[75] and the human decidua and placenta,[76] indicating the existence of a unique physiologic system in utero which may account for the complexities and uncertainties surrounding vitamin D metabolism in the fetoplacental unit.

Transition to neonatal life. At birth, the fetus-cum-neonate is certainly hypercalcemic, probably hypercalcitonemic, and perhaps hypoparathyroid. With placental separation, the previously abundant supply of calcium ceases abruptly and the infant's serum calcium

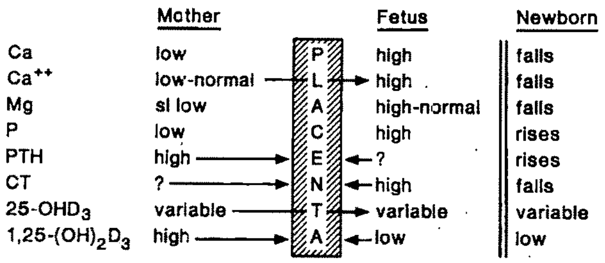

Fig. 3. Schematic model of calcium homeostasis in the pregnant woman, fetus, and newborn infant.

level begins to fall, reaching a nadir between 24 and 48 hours of age when it is about 1 mEq/L below that at birth. Thereafter, it rises slightly to stabilize at adult levels by 5 to 7 days of age. These changes involve both total and ionic forms of calcium.[77]

Phosphate levels fall slightly over the first day or two after birth but remain relatively high, and may even increase, during the first week of life. Changes in phosphorus are influenced strongly by dietary factors with higher values when feeding is delayed (because of tissue catabolism) or cow's milk is given. Magnesium exhibits considerable variation but generally remains fairly constant.

Endocrine factors are believed to play a major role in calcium homeostasis in the newborn infant. The traditional concept is that parathyroid suppression residual from late fetal life disappears gradually, ultimately permitting a progressive increase in parathyroid hormone output which first stabilizes and then raises the serum calcium level. However, as summarized earlier, the status of the fetal parathyroid is uncertain, and it therefore seems unlikely that simple suppression is responsible. The situation is likely to be much more complex, perhaps involving such factors as hypercalcitonemia[63, 78] or altered parathyroid hormone end-organ responsiveness[79] or factors related to 1,25-dihydroxyvitamin D_3 synthesis.[80, 81]

Prenatal factors influencing calcium homeostasis in the normal term newborn infant were examined by stepwise multiple regression analysis with the serum calcium levels at 24 hours of age as the dependent variable and a large number of clinical and laboratory characteristics entered as independent variables.[56] The only clinical variable of statistical significance was duration of gestation; in addition, six laboratory values (maternal parathyroid hormone and phosphorus, umbilical venous parathyroid hormone and magnesium, and neonatal magnesium and albumin) contributed significantly to the model. However, these seven independent variables in aggregate accounted for only 35% of the observed variation in neonatal serum calcium levels, implying that most of the fluctuation reflects factors operative after, as opposed to before, birth.

Early neonatal hypocalcemia, generally regarded as a fall below 3.5 mEq/L in total or 1.25 mEq/L in the ionic calcium level, is a relatively common neonatal complication, seen particularly with preterm birth,[82] maternal diabetes,[83] and birth asphyxia.[84] It is believed to represent an exaggeration of the normal pattern but, since the basis of the physiologic pattern is unclear, the pathophysiologic mechanism is also uncertain.

Summary of maternal-perinatal physiology

Fig. 3 summarizes the mechanisms of calcium homeostasis in the pregnant woman, fetus, and newborn infant. The principal maternal adjustment during pregnancy is increasing parathyroid hormone secretion which maintains the serum ionic calcium level within its characteristically narrow physiologic range in the face of extracellular fluid volume expansion, renal function increase, and placental calcium transfer. Calcitonin levels may or may not increase as well. Serum 25-hydroxyvitamin D_3 values are unaffected by pregnancy itself but 1,25-dihydroxyvitamin D_3 levels increase, perhaps as an effect of parathyroid hormone. The cardinal feature of calcium metabolism in the fetus is the active placental transport of large quantities of calcium, whereas parathyroid hormone and calcitonin do not cross the placenta. Theoretically, the resultant fetal hypercalcemia should suppress the fetal parathyroid and/or stimulate fetal calcitonin release; the latter is probably correct but evidence with respect to the former point is conflicting. 25-Hydroxyvitamin D_3 appears to cross the placenta freely but the placental permeability of 1,25-dihydroxyvitamin D_3 is questionable. With birth, the placental source of calcium terminates abruptly and the serum calcium level declines, perhaps aggravated by hypoparathyroidism and/or hypercalcitonemia residual from fetal life. After reaching a nadir between 24 and 48 hours of age, the neonatal calcium level stabilizes and then rises slightly to adult levels.

Clinical aspects

Maternal hyperparathyroidism. Primary hyperparathyroidism, a rare complication of pregnancy with fewer than 100 cases reported to date,[85-89] is usually due to parathyroid adenoma, although carcinoma has been observed on occasion.[90] The diagnosis should be suspected with the demonstration of persistently elevated serum calcium levels coupled with low serum and high urinary values of phosphorus. Parathyroid hormone values are usually elevated but may be within the normal range; in any event, however, they are inappropriate to the calcium level. In view of the physiologic adjustments of gestation, the diagnostic criteria need to be adjusted slightly in the pregnant woman (that is, lower serum total calcium and phosphorus and higher parathyroid hormone values). Gestation, with its increased calcium demands, may exacerbate primary hyperparathyroidism.[91]

Complications affect both mother and fetus-neonate. Maternal problems, particularly hyperemesis gravidarum, generalized weakness, renal calculi, pancreatitis, and psychiatric disorders, occur in at least a third of cases. Spontaneous abortion is more likely and fetal death in late gestation occurs with greatly increased frequency.

The most common neonatal complication is hypocalcemic tetany, reported in half or more of infants born to affected gravid women. In fact, the unexpected occurrence of neonatal tetany often provides the initial diagnostic clue to the maternal disease. The mechanism is generally regarded as involving prolonged fetal parathyroid suppression from chronic maternal-fetal hypercalcemia. However, this may be an overly simplistic view, for in some instances affected infants have been found to have normal or even elevated parathyroid hormone levels,[92, 93] suggesting that other factors (for instance, hypomagnesemia, end-organ refractoriness to parathyroid hormone, or defective 1,25-dihydroxyvitamin D_3 synthesis) may be involved.

The primary treatment of hyperparathyroidism is surgical and most authorities recommend prompt exploration once the diagnosis is made, even if the pregnant woman is asymptomatic.[85-88] A recent tabulation of reported experience[89] revealed a 26% pregnancy loss (abortion, stillbirth, or neonatal death) rate and a 33% frequency of neonatal calcium disturbance among 61 patients not operated upon compared with 6% and 0%, respectively, in 16 women who underwent parathyroidectomy during gestation. In one reported case, amniocentesis was done before and after operation; calcium levels fell by half and parathyroid hormone values doubles within a week after operation.[94] Recently, however, questions have been raised about the need for operation in asymptomatic cases and it has been suggested that conservative management may be preferable.[95, 96] As an alternative to surgery, oral phosphate therapy has been found to provide some degree of relief of maternal hypercalcemia.[97-99]

Maternal hypoparathyroidism. Hypoparathyroidism, usually resulting from inadvertent parathyroidectomy with thyroid surgical procedures, is relatively rare. The principal fetal effect, observed with both hypoparathyroidism and pseudohypoparathyroidism in the pregnant woman, is secondary hyperparathyroidism and bone demineralization.[100-103] Maternal treatment involves supplemental calcium and vitamin D, typically in daily doses of 3 to 5 gm and 100,000 IU, respectively. With the recent availability of 1-25-dihydroxyvitamin D_3 or calcitriol, this agent has been used with good results; dosages usually need to be increased (from 0.5 to 1 μg/day to 2 to 3 μg/day) to maintain the maternal serum calcium level with advancing gestation.[104, 105]

Concern has been expressed in the past about possible adverse fetal effects of large doses of vitamin D during pregnancy. Administration of $\geq$10,000 IU daily to pregnant rabbits caused hypercalcemia and supravalvular aortic stenosis[106] but no such effect has been reported in humans. In one case of vitamin D–resistant rickets, a pregnant woman was given 17 to 36 μg of 1,25-dihydroxyvitamin D_3 daily (approximately 10 times the customary dose); her infant had high blood levels but was otherwise normal.[107]

Heparin-induced osteopenia. Long-term use of heparin in the pregnant woman for treatment of thromboembolic or other disease has led to maternal osteopenia in some cases. The mechanism apparently involves a lowering of 1,25-dihydroxyvitamin D_3 levels by inhibiting renal 1α-hydroxylation of 25-hydroxyvitamin D_3.[108, 109] Thus, it may be that the patient on a regimen of long-term heparin therapy should be treated with supplemental calcitriol.

Diabetes mellitus. The well-known association between maternal diabetes and early neonatal hypocalcemia suggests that something about the metabolic state in the diabetic gravid woman affects the fetus in such a way as to impair its calcium homeostatic mechanism in the first few hours after birth.

Heath et al.[110] found no differences in calcium or calcitropic hormone levels with diabetes in nonpregnant subjects. However, Cruikshank et al.[111] reported that diabetic gravid women exhibited persistently low serum magnesium levels throughout most of gestation and failed to demonstrate the normal increase in parathyroid hormone levels; total and ionic calcium values were unaffected. These same investigators, in another study,[112] noted parathyroid hormone and calcium in cord blood and neonatal calcium at 24 hours all to be lower in diabetic women compared with normal ones. They hypothesized a state of chronic maternal hypomagnesemia leading to relative hypoparathyroidism in both mother and fetus with resultant propensity to hypocalcemia. Consistent with this theory are reports from other sources that infants of diabetic mothers are hypomagnesemic as well as hypocalcemic and that magnesium treatment corrects both abnormalities.[84, 113, 114]

Also consistent, in a negative way, is the finding that vitamin D administration to newborn infants prevents hypocalcemia with birth asphyxia but not with maternal diabetes.[115]

A different suggestion regarding hypocalcemia with maternal diabetes is that it involves hypoglycemia-stimulated glucagon release which in turn stimulates calcitonin secretion.[116]

Hypertension. Considerable current interest in hypertension in general involves a possible relationship with calcium. Belizan and Villar[117] and Villar et al.[118] have examined this matter with respect to preeclampsia and have made several interesting observations. Using epidemiologic data, they pointed out that the incidence of eclampsia varies inversely with calcium intake. In experimental studies, they observed further that calcium-deprived rats exhibited blood pressure rises during gestation[119] and that normal women given supplemental calcium had lower arterial pressures than unsupplemented gravid women.[120]

Low serum ionic calcium levels have been noted in nonpregnant subjects with hypertension,[121] and a study of calcium metabolism in pregnant women with essential hypertension found lower ionic calcium and parathyroid hormone levels and higher phosphorus values in comparison with values in gestational age–matched control subjects.[122] Another investigation, however, found no differences in total or ionic calcium or phosphorus with respect to either acute or chronic hypertensive disorders of pregnancy.[123]

Regardless of any possible pathogenetic role for calcium in pregnancy-related hypertension, the standard treatment for acute preeclampsia-eclampsia—magnesium sulfate—has certain clear implications for calcium homeostasis. Several studies by Cruikshank et al.[124, 125] indicate that intravenous infusion at customary doses (4 gm loading dose followed by 1 to 2 gm/hr) raises the maternal serum magnesium level by approximately 150% and lowers serum calcium by an average of 16%,[124] the latter as the result of increased urinary excretion.[125] Symptomatic maternal hypocalcemia, however, occurs rarely,[126] probably because maternal parathyroid hormone secretion increases markedly.[124] Infants born to magnesium-treated women are hypomagnesemic and hypocalcemic at birth but less so than their mothers because of a protective effect of the placenta.[124] Excess magnesium is cleared by the infant over 48 hours and the risk of hypocalcemia is not increased.[127] Breast-feeding is not contraindicated by maternal magnesium therapy.[128]

Dietary conditions. A number of studies have demonstrated an apparent benefit of calcium and/or vitamin D supplementation on growth,[129] bone density,[130, 131] and calcium homeostasis[132-134] in the fetus and newborn infant. These studies typically involved Asian women in whom a low dietary intake combined with clothing practices results in a marginal vitamin D status. Whether routine dietary supplementation confers any benefit in other populations is unclear.

The occurrence of leg cramps in the pregnant woman has been believed by some to reflect transient hypocalcemia due to low calcium and/or high phosphate intake and supplemental calcium (in the form of a nonphosphate salt) has been used widely. In a recent report, women with leg cramps randomly assigned to calcium therapy experienced symptomatic relief, but no significant effect was exhibited on serum ionic calcium levels.[135]

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BASIC SCIENCE SECTION

Placental prostacyclin production in normal and toxemic pregnancies

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Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation. Because toxemia is characterized by increased vasoconstriction frequently associated with increased platelet aggregation and reduced uteroplacental blood flow, a deficiency in prostacyclin production during pregnancy could contribute to the development of toxemia. Placentally produced prostacyclin could have both local effects on the uteroplacental vasculature and systemic effects because prostacyclin, unlike the other prostaglandins, is not extensively metabolized by the lungs. Fresh human term placentas were obtained immediately after delivery from 12 normal and 12 toxemic (blood pressure $\geq$140/90 mm Hg, urinary protein >0.3 gm/24 hours) pregnancies. Tissues (300 mg) were incubated in a sterile manner in 5 ml of Dulbecco's Modified Eagle's Medium for 48 hours at 37° C with 95% oxygen and 5% carbon dioxide in a metabolic shaker. Samples were collected at 8, 20, 32, and 48 hours, and analyzed for prostacyclin by radioimmunoassay of its stable metabolite, 6-keto-prostaglandin $F_{1\alpha}$. Prostacyclin production was significantly decreased in toxemic placental tissue compared with normal placental tissue (2.72 ± 0.49 versus 7.22 ± 0.44 pg/mg/hr, mean ± SE, $p < 0.01$). In both normal and toxemic placentas, prostacyclin production was inhibited by indomethacin (5 or 50 μmol/L) and not affected ($p > 0.10$) by arachidonic acid (5 or 100 μmol/L). Lowering the oxygen concentration from 95% to 20% significantly ($p < 0.01$) decreased prostacyclin production in normal but not toxemic placentas. Prostacyclin production rates in the amnion and chorion were not affected ($p > 0.10$) by toxemia. The amniotic and chorionic prostacyclin production rates were not different from each other ($p > 0.10$) and were only one seventh of the normal placental production rate. These data indicate that placental prostacyclin production is decreased in toxemia; therefore, this vasoactive prostaglandin may be involved in the causation and the associated hypertension and coagulation abnormalities of this disorder. (AM J OBSTET GYNECOL 1985;151:110-5.)

Key words: Placenta, prostacyclin, toxemia, pregnancy, hypertension

Toxemia is characterized by increased vasoconstriction frequently associated with increased platelet aggregation and reduced uteroplacental blood flow.[1, 2] Because prostacyclin is a potent vasodilator and an inhibitor of platelet aggregation,[3] defective prostacyclin production could contribute to the development of toxemia.

During normal pregnancy the production of prostacyclin is increased because maternal plasma concentrations of its stable metabolite, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), and its urinary metabolites are higher during late pregnancy than during early pregnancy or the nonpregnant state.[4, 5] In pregnancy-induced hypertension, prostacyclin production is apparently decreased but production rates have not been critically examined. Urinary metabolite concentrations of prostacyclin are depressed in toxemic pregnancies.[5] Prostacyclin-like activity in amniotic fluid is also decreased[6] as are the concentrations of amniotic fluid 6-keto-$PGF_{1\alpha}$.[7] Studies examining the production of prostacyclin by vascular tissue also suggest that decreased prostacyclin production is involved in toxemia.[8-11] Plasma levels of prostacyclin have been reported to be either decreased[12] or unchanged.[13]

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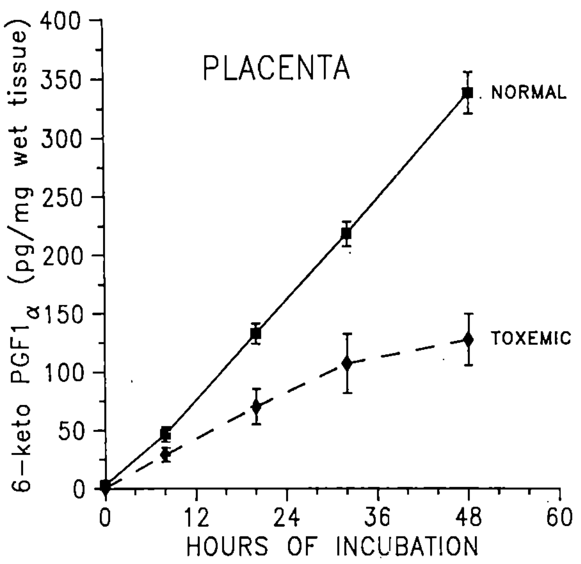

Fig. 1. Production rates of prostacyclin by normal and toxemic placental tissue, as estimated by the concentrations of its stable metabolite, 6-keto-$PGF_{1\alpha}$, in the incubation media. Normal versus toxemic production rate = 7.22 ± 0.44 versus 2.72 ± 0.49 pg/mg of wet tissue per hour, $p < 0.01$. Data represent mean ± SE (n = 12).

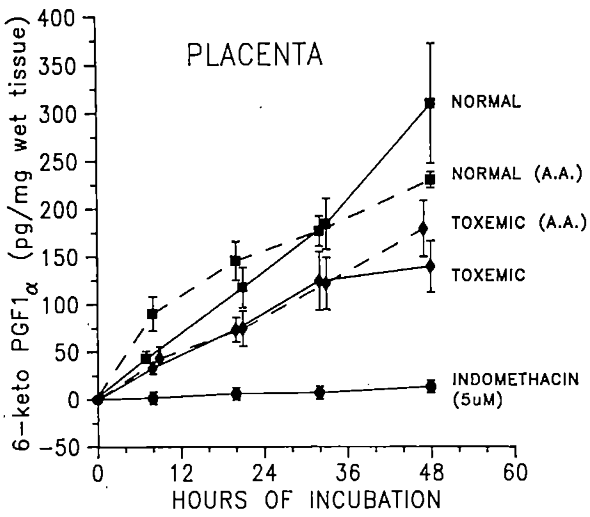

Fig. 2. Effects of indomethacin (5 μmol/L) (n = 4) and arachidonic acid (5 or 100 μmol/L) on prostacyclin production by normal (n = 4) and toxemic (n = 5) placental tissue. Prostacyclin production was significantly ($p < 0.01$) inhibited by indomethacin but not affected ($p > 0.10$) by arachidonic acid in either normal or toxemic placentas. Data represent mean ± SE.

The source of the additional prostacyclin during normal pregnancy is not known, but because the placenta is unique to pregnancy and produces prostacyclin[14] it is likely that it is responsible. Since prostacyclin is not extensively metabolized by the lungs, as are the other prostaglandins,[15] placentally produced prostacyclin could have both local and systemic effects. Because placental production of prostacyclin has not been examined in toxemia, this study was undertaken.

Material and methods

Term placentas were obtained within 15 minutes of delivery from 12 healthy, normotensive women and from 12 women with clinically diagnosed toxemia. A mixture of vaginal and cesarean deliveries was included in each group. The following criteria were used for the normal pregnancies: (1) maternal blood pressure (systolic/diastolic) ≤110/70 mm Hg; (2) no proteinuria; (3) gestational length between 36 and 43 weeks; (4) labor duration <18 hours for primigravid women and <12 hours for multigravid women; (5) no overt medical complications, such as diabetes, chronic infection, or metabolic, endocrine, nutritional, or hematologic complications; (6) no neonatal abnormalities, such as developmental defects, abnormal Apgar scores (1- and 5-minute tests), or birth weights <2.5 kg or >4.5 kg; (7) no third-trimester bleeding; (8) no gross placental abnormalities; (9) no positive findings at high-risk antenatal assessment; (10) No oligohydramnios or polyhydramnios; (11) no or minimal alcohol consumption or smoking during pregnancy. The same criteria were used to assess the toxemic pregnancies with the addition of two requirements: (1) maternal blood pressure (systolic/diastolic) ≥140/90 mm Hg on two separate readings 6 hours apart and (2) proteinuria, with a urinary protein level >0.3 gm per 24-hour urine collection.

Placental tissues were immediately chilled and processed under strict sterile conditions at 4° C. Placental, amniotic, and chorionic tissues were separated, minced and washed repeatedly with buffer. Excess buffer was removed with filter paper. Each tissue (300 mg) was incubated in quadruplicate in 5 ml of Dulbecco's Modified Eagle's Medium (Gibco, Grand Island, New York) for 48 hours at 37° C in a Dubnoff metabolic shaker. Tissues were oxygenated with 95% oxygen and 5% carbon dioxide. Some tissues were also gassed with a lower mixture of oxygen, that is, 20% oxygen, to assess the effect of the oxygen tension on prostacyclin production. Partial pressures and hydrogen ion concentrations in the incubation media were consistent at approximately $Po_2 = 250$ mm Hg, $Pco_2 = 20$ mm Hg, and pH = 7.50. Reducing the oxygen concentration to 20% reduced the Po_2 to approximately 70 mm Hg. Indomethacin and arachidonic acid were obtained from Sigma Chemical Company, St. Louis, Missouri.

Samples (120 μl) were collected at time 0 and at 8, 20, 32, and 48 hours of incubation. The production of prostacyclin was estimated by determining the concentrations in the incubation media of one of its stable metabolites, 6-keto-$PGF_{1\alpha}$. A highly specific radioimmunoassay was used. Briefly, the assay consisted of competitive binding of radioactive and natural 6-keto-$PGF_{1\alpha}$ to a highly specific antibody. Dextran-coated

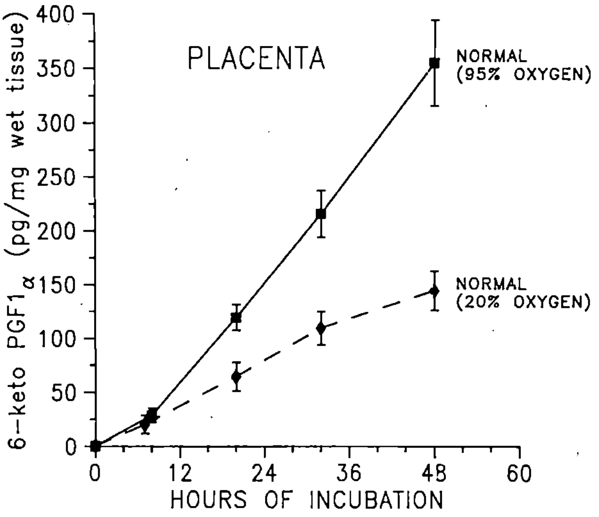

Fig. 3. Production rate of prostacyclin by normal placental tissue gassed with 95% or 20% oxygen. The production rate was significantly lower ($p < 0.01$) in tissue gassed with 20% oxygen. Data represent mean ± SE ($n = 5$).

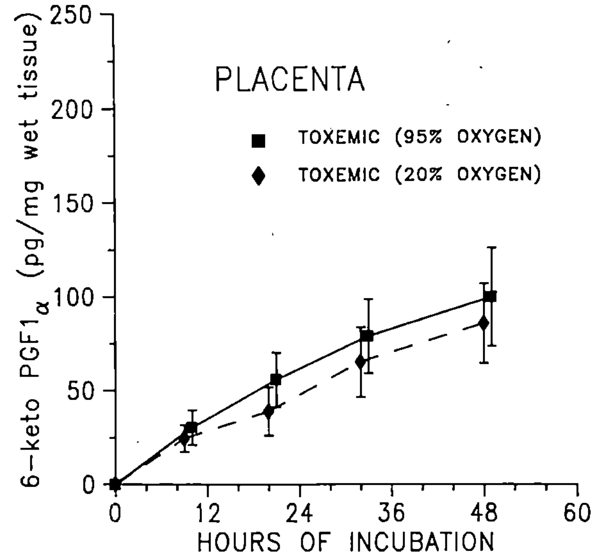

Fig. 4. Production rate of prostacyclin by toxemic placental tissue gassed with 95% or 20% oxygen. The production rates were not significantly different ($p > 0.10$). Data represent mean ± SE ($n = 5$).

charcoal was used to separate the bound fraction from the free fraction. The antibody was obtained from Seragen, Inc., Boston, Massachusetts, and radioactive 6-keto-$PGF_{1\alpha}$ from New England Nuclear, Boston, Massachusetts. Cross-reactivity of the antibody with other possible interfering eicosanoids was: prostaglandin $F_{1\alpha}$, 7.8%; 6-keto-prostaglandin E_1, 6.8%; prostaglandin $F_{2\alpha}$, 2.2%; prostaglandin E_1, 0.7%; prostaglandin E_2, 0.6%; prostaglandins D_2, A_2, A_1, B_1, and B_2, thromboxane B_2, 15-keto-prostaglandin $F_{2\alpha}$, 15-keto-prostaglandin E_2, 13,14-dihydro-15-keto-prostaglandin E_2, and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, all <0.01%. Within-assay variation was 7.5% and between-assay variation was 11.9%. The least detectable concentration was 20 ± 5 pg and 50% binding of the standard curve was 131 ± 5 pg (mean ± SE, $n = 8$). Serial sample dilutions were parallel to the standard curve (slope comparisons: $p > 0.10$) and addition of increasing amounts of 6-keto $PGF_{1\alpha}$ to Dulbecco's Modified Eagle's Medium resulted in a linear dose response ($r^2 = 1.00$, $b = 0.87$, $a = 0.01$). Dulbecco's Modified Eagle's Medium alone resulted in a zero dose response.

Tissue viability after 48 hours of incubation was assessed by trypan blue exclusion staining of trypsinized dispersed cells. Cells were dispersed with a 0.25% trypsin–ethylenediaminetetra-acetic acid solution (Gibco) for 60 minutes under constant agitation at 37° C with 95% oxygen and 5% carbon dioxide. After centrifugation at 100 × g for 15 minutes, the cellular pellet was resuspended in 0.5 ml of 0.1 mol/L of phosphate-buffered saline with 120 μl of a 0.4% solution of trypan blue (Gibco). After 5 minutes incubation, the cells were separated by centrifugation, resuspended in 0.5 ml of phosphate-buffered saline, and examined microscopically for cell membrane integrity and exclusion of dye. Differences between normal and toxemic placental hormone productions were statistically assessed by linear regression and analysis of variance with the Student-Newman-Keuls test.[16] Data were expressed as picograms per milligram of wet tissue per hour.

Results

There were no significant differences ($p > 0.10$) between normal and toxemic pregnancies for placental weights (544 ± 37 versus 521 ± 53 gm, respectively, mean ± SE, $n = 12$), infant weights (3.55 ± 0.17 versus 3.04 ± 0.14 kg), weeks of gestation at delivery (40 ± 1 versus 39 ± 1), age of the mothers (24 ± 2 versus 26 ± 2 years), weight gain of the mothers (31 ± 5 versus 38 ± 6 pounds), and Apgar scores of the infants (9 ± 1 versus 9 ± 1). All infants were healthy. Tissue viability as assessed by trypan blue exclusion staining was >95% after 48 hours of incubation in both the normal and the toxemic groups. In each group there were eight vaginal deliveries and four cesarean sections with two of the patients not yet in labor. The time in labor for the other women was 6.7 ± 0.9 versus 8.8 ± 1.2 hours (normal versus toxemia, $n = 10$, $p > 0.10$). Time in labor was not correlated with prostacyclin production ($r = 0.002$). Four of the normal patients and 11 of the toxemic patients were primigravid. Three of the toxemic patients had severe toxemia (systolic/diastolic ≥170/110 mm Hg.)

The production of prostacyclin by normal placental

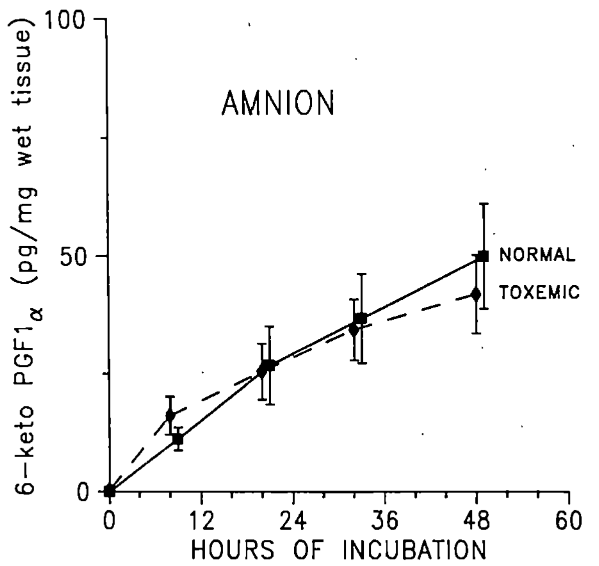

Fig. 5. Amniotic production rate of prostacyclin by normal and toxemic tissue. Normal versus toxemic production rates were not significantly different (0.98 ± 0.29 versus 0.82 ± 0.17 pg/mg of wet tissue per hour, $p > 0.10$). Data represent mean ± SE (n = 8).

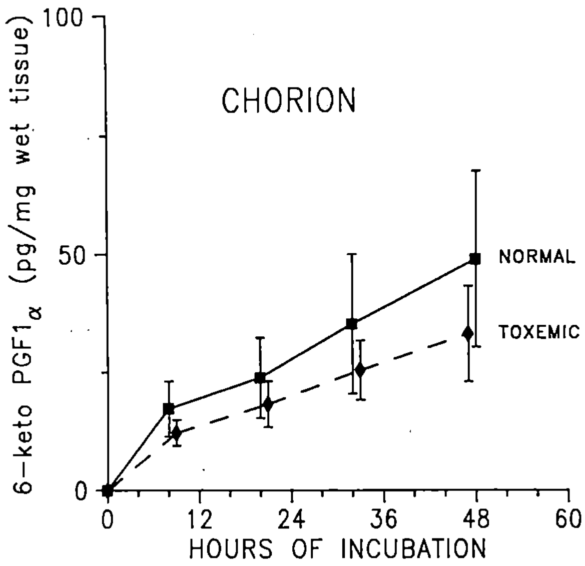

Fig. 6. Chorionic production rate of prostacyclin by normal and toxemic tissue. Normal versus toxemic production rates were not significantly different (0.95 ± 0.38 versus 0.69 ± 0.21 pg/mg of wet tissue per hour, $p > 0.10$). Data represent mean ± SE (n = 8).

tissue, as estimated by 6-keto-PGF$_{1\alpha}$, is shown in Fig. 1. The concentrations of 6-keto-PGF$_{1\alpha}$ in the incubation media increased progressively and in a linear fashion during the 48 hours of incubation. They were significantly elevated at 8 hours, and again at 20, 32, and 48 hours ($p < 0.01$). Peak levels after 48 hours averaged 346 pg/mg. The production rate was 7.22 ± 0.44 pg/mg/hr (mean ± SE, n = 12) or approximately 94 μg/day.

In comparison to production by normal tissue, the production of prostacyclin by toxemic placentas was considerably depressed (Fig. 1). The concentrations of 6-keto-PGF$_{1\alpha}$ were significantly lower than those from normal placentas at 8, 20, 32, and 48 hours ($p < 0.01$). Peak levels at 48 hours averaged only 128 pg/mg compared to 346 pg/mg for normal placentas. The production rate for the toxemic placentas was only 2.72 ± 0.49 pg/mg/hr (mean ± SE, n = 12), and this was significantly less than the production rate of the normal placentas of 7.22 pg/mg/hr ($p < 0.01$). Whitish infarcted areas in some of the toxemic placentas were also separated and incubated. The infarcted tissue also produced prostacyclin (4.01 ± 0.38 pg/mg/hr, n = 4).

To assure we were measuring prostacyclin production rather than tissue release and to assure that our production rates were not limited as the result of precursor availability, some tissues were incubated in Dulbecco's Modified Eagle's Medium with varying concentrations of indomethacin or arachidonic acid. Complete inhibition of prostacyclin production was produced by 50 μmol/L of indomethacin, whereas 5 μmol/L of indomethacin allowed a slight amount to be produced (0.27 ± 0.14 pg/mg/hr, n = 4) (Fig. 2). Addition of arachidonic acid (5 or 100 μmol/L) did not significantly affect prostacyclin production ($p > 0.10$) in either normal or toxemic placentas (Fig. 2). There was no difference between the two dose levels. The production rates in normal placentas with and without arachidonic acid were 4.38 ± 0.19 versus 6.33 ± 1.26 pg/mg/hr (n = 4), and those in toxemic placentas were 3.62 ± 0.63 versus 3.02 ± 0.68 pg/mg/hr (n = 5), respectively.

To assess the requirement of oxygen for prostacyclin production, we gassed some tissues with a lower percentage of oxygen. Reducing the oxygen concentration from 95% to 20% significantly decreased prostacyclin production in normal placentas (7.55 ± 0.77 versus 3.09 ± 0.39 pg/mg/hr, n = 5, $p < 0.05$) but not in toxemic placentas (2.08 ± 0.54 versus 1.80 ± 0.45 pg/mg/hr, n = 5, $p > 0.10$) (Figs. 3 and 4).

Prostacyclin production rates in the amnion and chorion were not affected ($p > 0.10$) by toxemia (Figs. 5 and 6). Production rates in the amnion in normal versus toxemic pregnancies were 0.98 ± 0.29 versus 0.82 ± 0.17 pg/mg/hr, n = 8 and in the chorion were 0.95 ± 0.38 versus 0.69 ± 0.21 pg/mg/hr, n = 8. Amniotic and chorionic prostacyclin production rates were not significantly different from each other ($p > 0.10$), but they were significantly less than the placental production rates ($p < 0.01$). For example, in the normal pregnancies the amnion or chorion produced only one seventh as much prostacyclin as the placenta.

Comment

Prostacyclin production by toxemic placentas was significantly less than that produced by normal placentas (Fig. 1). The toxemic production rate was approximately one third the normal production rate. Because prostacyclin is a potent vasodilator and an inhibitor of platelet aggregation,[3] defective production by the toxemic placenta could contribute to the clinical manifestations of this disorder. Placentally produced prostacyclin could have both local and systemic effects because it is not extensively metabolized by the lungs.[15]

Other than prostacyclin production and the clinical signs of toxemia, the normal and toxemic groups were similar. There were no significant differences between normal and toxemic pregnancies for placental weights, infant weights, weeks of gestation at delivery, number of vaginal versus cesarean section deliveries, time in labor, age of the mothers, weight gain of the mothers, Apgar scores of the infants, and tissue viability. All infants were healthy. The presence of more areas of infarction in the toxemic placentas compared to the normal placentas also cannot account for the decreased prostacyclin production in toxemia because these areas also produced prostacyclin. Therefore, the decreased prostacyclin production by the toxemic placentas is a result of or a cause of the disorder and not due to nonspecific factors.

To establish that the concentrations of 6-keto-PGF$_{1\alpha}$ in the incubation media represented prostacyclin production and not release, we added indomethacin to the incubation media. In each case indomethacin inhibited any increase in 6-keto-PGF$_{1\alpha}$ (Fig. 2). Therefore, concentrations of 6-keto-PGF$_{1\alpha}$ in the incubation media represent prostacyclin production and not release.

Studies were also done to determine if decreased prostacyclin production in toxemia is related to precursor availability and to determine if sufficient precursor is available in the placenta to sustain prostacyclin production during 48 hours of in vitro incubation. Addition of arachidonic acid to the incubation media initially enhanced prostacyclin production, probably because the arachidonic acid in the media was readily available for conversion, but it did not substantially affect prostacyclin production over the 48-hour period of incubation in either normal or toxemic placentas (Fig. 2). Therefore, precursor availability is not a limiting factor and cannot account for the difference in prostacyclin production by normal and toxemic placentas.

Because prostacyclin synthase requires oxygen to convert prostaglandin endoperoxide to prostacyclin,[17] we examined prostacyclin production in two different oxygen environments. Reducing the oxygen concentration from 95% to 20% significantly decreased prostacyclin production in normal tissue but not in toxemic tissue (Figs. 3 and 4). This suggests that defective prostacyclin production in toxemia may be due in part to limited availability of the prostacyclin-forming enzyme, that is, lower oxygen concentration was sufficient to activate all of the prostacyclin synthase present in the toxemic placental tissue but not in the normal placental tissue.

Studies examining the production of prostacyclin solely by vascular tissue also indicate that decreased prostacyclin production is involved in toxemia. In 1980, Remuzzi et al.[8] using a bioassay reported that prostacyclin production was decreased in umbilical arteries and placental veins obtained from five patients with severe toxemia. Bussolino et al.[9] also used a bioassay to examine vascular prostacyclin production in vessels obtained from normal and toxemic pregnancies. They found that prostacyclin production was decreased in placental veins, subcutaneous vessels, and uterine vessels in severely toxemic patients. Carreras et al[11] used a radioimmunoassay to measure 6-keto-PGF$_{1\alpha}$ and found its production decreased by vascular fragments of the umbilical artery and placental veins of hypertensive women. Downing et al.[10] examined the enzymatic kinetics of prostacyclin synthase in umbilical arteries. They found that the enzyme activity was the same in cords of normal and hypertensive pregnancies but the cords of hypertensive pregnancies contained less enzyme.

Amniotic and chorionic production of prostacyclin did not differ between normal and toxemic tissues (Figs. 5 and 6). The production rates were approximately one seventh the production rate of normal placental tissue. This is in contradistinction to the findings of Mitchell et al.[14] who used a superfusion technique to investigate prostacyclin production by conceptual tissues. They found that the amnion produced twice as much prostacyclin as either the chorion or the placenta. Methodologic differences might account for the discrepancy between the two studies. It seems reasonable to us, however, that placental tissue would produce more prostacyclin than either the amnion or the chorion because the placenta is a highly vascularized organ and preliminary immunocytofluorescent studies suggest that both endothelial and syncytiotrophoblastic tissues produce prostacyclin (Walsh SW, et al., unpublished data).

It was previously suggested that defective prostaglandin production or a loss of response to prostaglandins contributes to the development of toxemia.[1, 18, 19] The work of Gant et al.[20] and of Everett et al.[19] provides support for the hypothesis that defective production of a vasodilatory prostanoid is involved in toxemia. Gant et al. demonstrated that the dose of angiotensin II required to elicit a pressor response of 20 mm Hg in diastolic pressure was significantly less in midgestational women destined to develop hypertension than in women with normal pregnancies. Everett et al. then showed that the dose of angiotensin II necessary to cause a 20 mm Hg rise in diastolic pressure in women with normal pregnancies was significantly reduced in women treated with prostaglandin synthetase inhibitors. Therefore, the increased vascular responsiveness of toxemia was mimicked by producing defective prostaglandin synthesis.

An imbalance in the production of prostacyclin and thromboxane could account for the major clinical symptoms of toxemia. Placental thromboxane production is increased in toxemia[21]; therefore, the reduced amount of prostacyclin would not effectively antagonize the vasoconstrictor and platelet-aggregating actions of the increased amount of thromboxane. Furthermore, with less prostacyclin, the vasoconstrictor effects of angiotensin II and catecholamines would also not be efficiently opposed. The renin-angiotensin system is suppressed in toxemia but angiotensin II levels are still elevated over those of the nonpregnant state.[1,22] Prostacyclin stimulates this system[23,24] so defective prostacyclin production might also explain why angiotensin II levels are lower in toxemia than in normal pregnancy.

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Influence of a vitamin E–deficient diet on prostacyclin production by mesometrial triangles and aortic rings from nondiabetic and diabetic pregnant rats

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We investigated the effect of a vitamin E–deficient diet on the synthesis of prostacyclin in nondiabetic and diabetic pregnant rats. In both groups the diet reduced fetal growth and prostacyclin production by mesometrial triangles; it also increased serum lipid peroxides, which are known to inhibit prostacyclin synthesis. Independently of the diet, mesometrial triangles of diabetic rats produced more prostacyclin than those of control rats, whereas an opposite tendency was found in the aortic rings. Our model seems useful for fundamental and pharmacologic studies of the vascular pathology of pregnancy. (AM J OBSTET GYNECOL 1985;151:116-20.)

Key words: Prostacyclin, vitamin E, pregnancy, diabetes

Prostacyclin, a prostanoid mainly generated in vascular endothelial cells, is a potent vasodilator and the strongest natural inhibitor of platelet aggregation. It is thought to play an important role as modulator of the hemodynamic changes that characterize normal pregnancy and as a local defense mechanism against thrombus formation. Decreased prostacyclin formation has been observed in several pathologic conditions of pregnancy in the human, including diabetes and preeclampsia.[1]

Pregnant rats fed a vitamin E–deficient diet develop some symptoms found in human eclamptic disease.[2] The aim of this study was to investigate whether this animal model is associated with impaired prostacyclin production. We concentrated on prostacyclin generation by mesometrial triangles, since these are identifiable highly vascularized structures, strategically situated in the mesometrium associated with the developing placentas, comparable to the maternal part ("the placental bed") of the human placenta (Fig. 1).

Material and methods

Animals, dietary regimen, and classification. Wistar rats (±200 gm) were mated and divided into four groups. Groups I and III received a standard laboratory diet (RMH-GS, Hope Farms). Groups II and IV were fed the experimental diet, containing 58% cornstarch, 18.5% purified casein, 9.5% brewers' yeast, 4% salt mixture (Hope Farms), and 10% cod-liver oil. The experimental diet was made rancid by thoroughly mixing the ingredients, spreading it over a large surface, and keeping it at room temperature for 72 hours. The small amount of tocopherol present is assumed to be rendered ineffective by the oxidative action of the highly unsaturated fatty acids in cod-liver oil, after exposure to air. The food was then stored at −20° C to minimize further oxidative changes. Portions of the diet were removed from the freezer daily and fed ad libitum from day 1 of pregnancy on. Day 1 of gestation was determined by the presence of the copulation plug. Fresh water was supplied at all times.

Groups I and II consisted of nondiabetic animals; groups III and IV were made diabetic by intravenous injection of 50 mg streptozocin (Zanosar, Upjohn) per kilogram of body weight, 1 day after copulation. We finally obtained 14, 14, 17, and eight pregnant animals in groups I, II, III, and IV, respectively.

Determination of prostacyclin production by mesometrial triangles and aortic rings. On day 22, one day before the expected natural end of their pregnancies, a blood sample was taken from the abdominal aorta, and the rats were put to death after pentobarbital anesthesia (60 mg/kg of body weight intraperitoneally).

Three mesometrial triangles per animal and the total thoracic aorta were rapidly excised. Following removal of the adventitial layer, the aortas (15.9 ± 6.7 mg, n = 53) were divided into halves: one half was further cut into four equally sized rings, the other was con-

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served for tissue studies. The mesometrial triangles and aortic rings were incubated in 2.5 ml Tris-buffer (0.05 mmol/L, pH 7.4) at room temperature on a rocker platform; 100 µl samples were withdrawn at 0, 4, 8, 12, 16, 20 and 24 minutes after starting incubation and kept at least 1 hour at room temperature in order to obtain a complete degradation of prostacyclin to 6-keto-prostaglandin $F_{1\alpha}$. They were then stored at −24° C. Upon completion of the experiment, all samples were assayed for 6-keto-prostaglandin $F_{1\alpha}$ by a specific radioimmunoassay as previously described.[3]

For each mesometrial triangle and the four aortic rings, a linear regression line was calculated for the 6-keto-prostaglandin $F_{1\alpha}$ values as a function of time. The regression line then determines the prostacyclin production per time unit. The mean correlation coefficient between time and 6-keto-prostaglandin $F_{1\alpha}$ in all our experiments was 0.95 ($p < 0.005$). Number and weights of fetuses, placentas and mesometrial triangles were carefully noted.

Plasma values. Plasma glucose levels were assayed by the glucose oxidase technique (Beckman automatic glucose analyzer II) and plasma progesterone by a radioimmunoassay. Lipid peroxides in plasma were determined as malondialdehyde by reaction with thiobarbituric acid.[4] Ultraviolet fluorescence of plasma at an emission wavelength of 395 nm after excitation at 325 nm, another index of lipid peroxidation, was measured on an Aminco SPF-500 spectrofluorimeter and expressed in arbitrary units.[5]

Differences between groups were analyzed for significance by use of Wilcoxon's test. Data are presented as mean ± SE. NS denotes differences that were not significant.

Results

In nondiabetic and diabetic animals the vitamin E–deficient diet produced less and significantly lighter fetuses but placentas with a normal weight (groups II and IV versus groups I and III, Table I).

The significant differences in weight of the pregnant animals in groups II and IV as compared with groups I and III disappeared after subtracting the contribution of the involved fetuses and placentas, and therefore were probably not due to a reduced intake of the vitamin E–deficient diet. The placentas of diabetic animals (0.532 ± 0.091 gm, n = 227) were significantly ($p < 0.005$) heavier than those of the nondiabetic groups (0.384 ± 0.039 gm, n = 317). Fetuses of both groups of diabetic rats (III and IV) were significantly ($p < 0.001$) lighter than those of the corresponding nondiabetic groups I and II.

The nonfasting glycemias (520.29 ± 84 mg/100 ml, n = 25) of the animals that received streptozocin (groups III and IV) were significantly ($p < 0.005$) higher than those (128 ± 13.06 mg/100 ml, n = 28) of the nontreated ones (groups I and II). The vitamin E–deficient diet did not influence the glycemia.

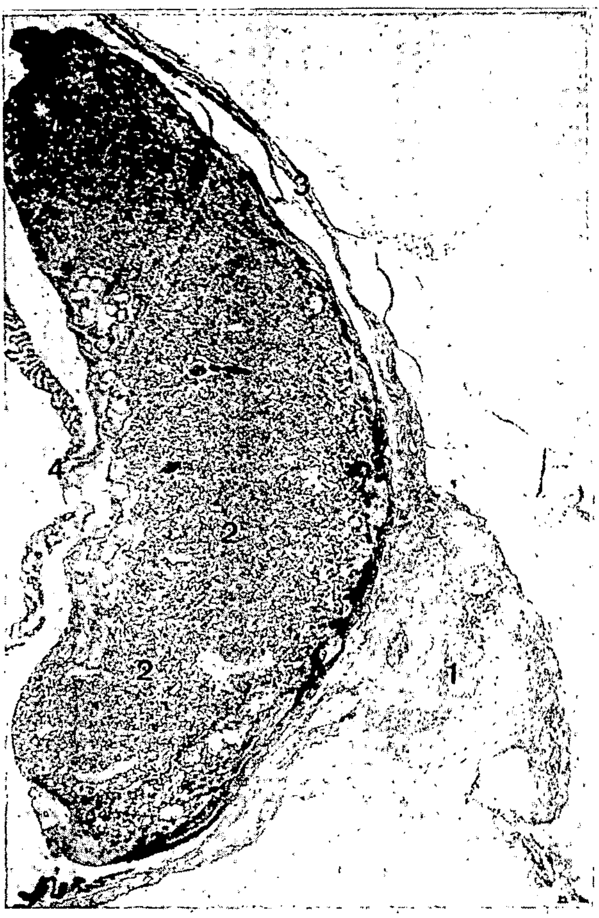

Fig. 1. Cross section of a mesometrial triangle and placenta. *1*, Mesometrial triangle; *2*, placenta; *3*, uterine wall; *4*, yolk sac.

The prostacyclin production by mesometrial triangles and by aortic rings is shown in Table II.

Mesometrial triangles of nondiabetic rats fed the vitamin E–deficient diet (group II) produced significantly less prostacyclin than those of the control rats (group I). Although aortic rings of nondiabetic rats (group II) and mesometrial triangles and aortic rings of diabetic rats fed the vitamin E–deficient diet (group IV) showed the same tendency, the differences did not reach a statistically significant level. Mesometrial triangles of diabetic rats (groups III and IV) produced significantly ($p < 0.0005$) more prostacyclin than those of the nondiabetic control rats (groups I and II). When the aortic rings were considered, an opposite but not significant trend was found.

Lipid peroxides determined by ultraviolet fluorescence and as malondialdehyde (Table III) were signif-

Table I. Weight evolution of the pregnant animals, number of fetuses per animal, and weight of fetuses and placentas

	Nondiabetic group		*Diabetic group*	
	I: standard diet	*II: vitamin E–deficient diet*	*III: standard diet*	*IV: vitamin E–deficient diet*
No. of rats	14	14	17	8
Weight of rats				
Day 1 (gm)	206.4 ± 6.2	206.5 ± 7.2	203.1 ± 5.9	208 ± 9.3
Significance	NS		NS	
Day 14 (gm)	268.7 ± 16	253.4 ± 14.5	231.2 ± 18.9	186 ± 12.6
Significance	$p < 0.05$		$p < 0.001$	
Day 21 (gm)	330 ± 20	311.6 ± 12.1	256.2 ± 22.9	223.6 ± 14.6
Significance	$p < 0.01$		$p < 0.05$	
Mean No. of fetuses per animal	12.4 ± 2.3	11.0 ± 2.8	9.0 ± 3.2	9.12 ± 2.7
Significance	NS		NS	
No. of fetuses	164	154	153	73
Mean weight				
Fetuses (gm)	4.551 ± 0.22	4.312 ± 0.237	3.646 ± 0.432	3.056 ± 0.270
Significance	$p < 0.01$		$p < 0.01$	
Placentas (gm)	0.388 ± 0.022	0.380 ± 0.051	0.544 ± 0.095	0.485 ± 0.065
Significance	NS		NS	
Mesometrial triangles (mg)	49.39 ± 7.24	44.16 ± 4.9	50.09 ± 10	48.94 ± 9.54
Significance	NS		NS	
Weight of rats on day 22 after correcting for fetal and placental contributions (gm)	268.3 ± 13.9	259.7 ± 10.3	219.11 ± 16.02	191 ± 8.49
Significance	NS		NS	

Table II. Prostacyclin production by mesometrial triangles and by aortic rings

	Nondiabetic group		*Diabetic group*	
	I: standard diet	*II: vitamin E–deficient diet*	*III: standard diet*	*IV: vitamin E–deficient diet*
Prostacyclin production by mesometrial triangles (pg/mesometrial triangle/min)	43.17 ± 17.33	31.66 ± 12.85	76.98 ± 54.59	59.21 ± 27.74
No. in group	42	42	51	24
Significance	$p < 0.025$		NS	
Prostacyclin production by aortic ring (pg/aorta/min)	1316 ± 624	1233 ± 503	1192 ± 625	1015 ± 381
No. in group	14	14	17	8
Significance	NS		NS	

icantly higher in the vitamin E–deficient groups II and IV, compared with the groups fed a standard diet (groups I and III). Diabetic animals (groups II and IV) showed consistently higher levels than the nondiabetic control animals (groups I and II).

As shown in Table IV, higher progesterone levels were found in group II than in group I. This argues against a general unselective toxic effect of the diet.

Comment

Nondiabetic and diabetic pregnant rats, fed an anti–vitamin E stress diet showed a decreased prostacyclin formation by their mesometrial triangles and increased plasma levels of lipid peroxides. The same tendency was found in the prostacyclin production by their aortas, and in fact, it had already been observed in nonpregnant animals fed a pure vitamin E–deficient diet for several months.[6] The addition of partially peroxided polyunsaturated fatty acids, which will further deplete the endogenous stores of tocopherol, and perhaps additionally the presence of pregnancy accelerate the impact of the diet. It was reported that the toxicity of the diet could be increased by augmenting the amount of oxidized fat and that the animals were effectively

Table III. Lipid peroxides determined by ultraviolet fluorescence and as malondialdehyde

	Nondiabetic group		*Diabetic group*	
	I: standard diet	*II: vitamin E–deficient diet*	*III: standard diet*	*IV: vitamin E–deficient diet*
Ultraviolet fluorescence	1.515 ± 0.716	2.707 ± 0.896	3.268 ± 1.071	9.044 ± 3.341
	$p < 0.001$		$p < 0.001$	
Malondialdehyde (nmol/ml)	2.133 ± 0.56	2.966 ± 0.666	1.466 ± 0.366	5.133 ± 2.433
	$p < 0.001$		$p < 0.001$	

Table IV. Mean plasma progesterone levels in the four groups

	Nondiabetic group		*Diabetic group*	
	I: standard diet	*II: vitamin E–deficient diet*	*III: standard diet*	*IV: vitamin E–deficient diet*
Plasma progesterone (ng/ml)	29.05 ± 9.12	51.68 ± 28.62	26.08 ± 12.92	24.00 ± 17.46
	$p < 0.005$		NS	

protected by vitamin E in adequate amounts.[2] Using a more oxidized diet (exposure to air for 120 instead of 72 hours), we have observed a more pronounced decrease of prostacyclin production.[7] In comparison with the aortas, the highly vascularized mesometrial triangles produced remarkably little prostacyclin. This could partially be explained by a reduced contact of the vessels with the incubation medium and by less damage than to the aortas cut into rings.

A vitamin E–deficient diet was originally proposed as a model for the study of eclamptic disease. Animals receiving such diet showed some clinical and pathologic changes (evidence of disseminated intravascular coagulation, placental separation with hemorrhage, intrauterine fetal death) that resemble those of preeclampsia.[2] Since they, however, did not become hypertensive, did not have increased proteinuria or weight changes indicative of edema, and had no swelling of the endothelial cells of the glomerular capillaries, decisive features of the human syndrome were not imitated.[8]

Multiple factors are involved in the pathogenesis of preeclampsia. Recently attention has been focused on decreased umbilical, uterine, amniotic, and fetal prostacyclin production.[1] An abnormal synthesis of vasodilator prostaglandins by uterus and vessels allows an explanation for diverse characteristics of preeclampsia: reduction of uteroplacental blood flow, enhanced uterine renin secretion, increased angiotensin II sensitivity, decreased renal renin secretion, and platelet aggregation.[9-11] It is also interesting to note that augmented levels of lipid peroxides and free radical peroxidation products have been reported in preeclamptic women.[5]

We demonstrated an impaired prostacyclin generation in this experiment model. If it also occurs in the human situation, it offers new possibilities of verifying hypothesized consequences of prostacyclin deficient states and of correcting the deficiency.

In pregnant diabetic rats, the prostacyclin production by mesometrial triangles was significantly increased as compared with production in the nondiabetic control animals, whereas an opposite but nonsignificant trend was found in aortic rings. Diminished aortic prostacyclin formation and augmented lipid peroxide levels have been documented in nonpregnant rats with streptozocin diabetes.[12] In our experimental model the vitamin E–deficient diet reinforced these alterations; conversely, a high vitamin E diet given to nonpregnant diabetic rats for 2 to 3 months has been shown to normalize thrombin-induced platelet thromboxane generation, vascular prostacyclin production, and lipid peroxide levels.[12] It has been suggested that in human diabetes an absolute or relative prostacyclin deficiency could trigger the onset of diabetic vascular complications.[13]

The different response between mesometrial triangles and aortas, similar to that already reported for lungs and glomeruli,[14, 15] may reflect the multiplicity of cell types and their enzymatic compositions. The physiologic meaning of this enhanced prostacyclin production by mesometrial triangles in diabetes is not clear, but since vasodilator prostaglandins seem to play a role in local blood flow regulation,[16] one could hypothesize that it contributes to the compensatory placental hypertrophy characterizing these diabetic pregnancies.

In conclusion, use of a vitamin E–deficient diet in pregnant rats makes it possible to impair prostacyclin generation, probably by an increase in free radical oxidation (peroxidation) products, which are known to inhibit prostacyclin synthetase. If in human obstetric pathologic conditions, such as preeclampsia, fetal growth retardation with and without hypertension, and diabetes, a decreased prostacyclin formation is a substantial finding, the model could be useful for fundamental and pharmacologic studies on some aspects of those conditions.

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6-Keto prostaglandin $F_{1\alpha}$, thromboxane B_2, and 13,14-dihydro-15-keto prostaglandin F concentrations of normotensive and preeclamptic patients during pregnancy, delivery, and the postpartum period

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Plasma 6-keto prostaglandin $F_{1\alpha}$, 13,14-dihydro-15-keto prostaglandin F, and thromboxane B_2 levels were measured in normotensive and preeclamptic patients during pregnancy and the postpartum period. From 30 to 40 weeks of gestation, 6-keto prostaglandin $F_{1\alpha}$ levels of preeclamptic patients were significantly lower than those of normotensive women; 13,14-dihydro-15-keto prostaglandin F and thromboxane B_2 concentrations in preeclamptic patients did not significantly differ from those of the normotensive group. At delivery, 6-keto prostaglandin $F_{1\alpha}$ levels of maternal and umbilical venous plasma of preeclamptic women were also significantly lower than those of normotensive women. In the postpartum period these three prostanoids were not significantly different in normotensive women compared to preeclamptic women, with clinical preeclamptic symptoms soon disappearing in most of our patients. From the results, it seems that prostacyclin plays an important role in preeclampsia. (AM J OBSTET GYNECOL 1985;151:121-7.)

Key words: Prostaglandins, preeclampsia, pregnancy

Since the discovery of prostacyclin[1] and thromboxane A_2, the physiologic roles of these two unstable prostanoids as well as their clinical significance in hypertension and other disorders have been discussed. Mitchell et al.[2] and Remuzzi et al.[3] described high prostaglandin I_2 production in fetal vessels, which suggests that it contributes to the maintenance of fetal circulation.

The etiologic involvement of prostanoids has been suspected in the development of the arteriole contraction and hypertension of preeclampsia. Remuzzi et al.[4-6] described depressed placental prostaglandin I_2 production in preeclamptic patients. But the role of prostaglandin I_2 in preeclampsia is still controversial. Tsukatani,[7] Massobrio et al.[8] and Lewis et al.[9] described lower 6-keto $PGF_{1\alpha}$ level in preeclamptic patients than in normal subjects. But Ylikorkala et al.[10] reported unchanged 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) levels, and Koullapis et al.[11] reported rather higher 6-keto $PGF_{1\alpha}$ concentrations in preeclamptic patients than in normotensive pregnants.

The purpose of this study was to determine not only 6-keto $PGF_{1\alpha}$ concentrations but also those of 13,14-dihydro-15-keto prostaglandin F (PGFM) and thromboxane B_2 (TXB_2) in maternal plasma, umbilical arterial, and venous plasma of normotensive and preeclamptic patients during pregnancy, delivery, and the postpartum period.

Material and methods

Patients. The cases were divided into three groups: 23 normotensive pregnant, 15 preeclamptic, and 13 postpartum groups. The 23 normotensive subjects, (13 primigravid and 10 multigravid) were at 19 to 41 weeks of gestation. The 15 preeclamptic patients included 10 primigravid and five multigravid women. The normotensive postpartum group was made up of 16 primigravid and seven multigravid women. Diagnosis of preeclampsia was based on blood pressure higher than 170/110 mm Hg and proteinuria with edema. Patients with other complications, such as diabetes mellitus, were excluded. No patient was administered drugs that were reported to affect prostaglandin metabolism or blood pressure. The normotensive group were delivered at term without any complications. Fetal weights ranged from 2600 to 3400 gm.

Blood collection. Blood was obtained from the antecubital vein in early morning after at least 1 hour of bed rest. Immediately after delivery, the umbilical cord was clamped at the distal and proximal portions. Um-

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Table IA. Cross-reactivity of antisera (%)

	Anti 6-keto $PGF_{1\alpha}$	*Anti* TXB_2
6-keto $PGF_{1\alpha}$	100	0.04
TXB_2	<0.05	100
PGE_1	4.0	0.07
PGE_2	4.6	0.05
$PGF_{1\alpha}$	2.2	0.4
$PGF_{2\alpha}$	2.5	0.2

Table IB. Cross-reactivity of antisera (%)

	Anti PGFM
13,14-dihydro-15-keto $PGF_{2\alpha}$	100
13,14-dihydro-15-keto $PGF_{1\alpha}$	179
13,14-dihydro $PGF_{2\alpha}$	1.2
15-keto $PGF_{2\alpha}$	25.4
13,14-dihydro-15-keto PGE_2	0.6
$PGF_{1\alpha}$	0.3
$PGF_{2\alpha}$	0.2

Table II. Prostaglandin concentration (pg/ml, mean ± SE) during pregnancy and postpartum period

Group	*6-keto* $PGF_{1\alpha}$	*PGFM*	TXB_2
Normotensive			
Pregnancy	318.6 ± 58.0*	145.7 ± 20.0*	56.6 ± 7.3*
Postpartum	298.1 ± 66.9	253.5 ± 21.4*	127.5 ± 28.4*
Preeclamptic			
Pregnancy	118.8 ± 16.4*	247.1 ± 48.5	119.5 ± 34.0
Postpartum	217.7 ± 62.0	490.0 ± 134.4	172.7 ± 91.9

*$p < 0.001$.

bilical venous blood was collected by puncture of the vessels. At the same time, maternal venous blood was collected.

Samples were placed in ice-cold test tubes containing 0.05 ml of 0.4% aspirin and 0.45 ml of 2% ethylenediaminetetra-acetate solution. Samples were stirred gently and centrifuged at 2500 rpm at 4° C for 15 minutes. Plasma was separated and frozen as soon as possible and stored at −20° C until extraction.

Reagents. Authentic prostanoids and their antisera were provided by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Radioactive prostanoids were purchased from Amersham International Plc (Buckinghamshire, England). Plastic thin-layer chromatography plates of Merck (DC-Plastikfolien Kieselgel 60, Merck, Darmstadt, West Germany) were used. Ethyl alcohol, acetic acid, and ammonia of ultra-fine grade were used. Other reagents used in extraction and purification were of guaranteed quality.

Extraction. After addition of 3000 dpm of radioactive tracer, plasma was acidified to pH 3 with hydrochloric acid. A threefold volume of ethyl acetate was added and mixed. The organic phase was stored and extraction was repeated three times. The stored organic phase was evaporated in vacuo at 40° C. Residue was dissolved in 15 ml of 0.05 mol/L of phosphate buffer containing 10% methyl alcohol; 20 ml of petroleum ether was added and mixed, and the organic phase was discarded. The remaining water phase was acidified to pH 3 again and reextracted with 20 ml of ethyl acetate. After removal of the water phase the resulting organic phase was evaporated and dissolved in a small amount of mixed solution of ethyl alcohol and ethyl acetate.

Separation. This solution was spotted on a plastic thin-layer chromatography plate and developed by the organic phase of ethyl acetate/water/2,2,4-trimethyl pentane/acetic acid (110 : 100 : 50 : 20, v/v/v/v). After iodine vapor staining, a corresponding area of each prostaglandin was scraped into a test tube. Each fraction of 6-keto $PGF_{1\alpha}$, PGFM, and TXB_2 was extracted four times with 2 ml of methyl alcohol containing 0.5% acetic acid. Methyl alcohol was evaporated under nitrogen stream, and the residue was dissolved in 0.9 ml of assay buffer for radioimmunoassay, which consisted of 0.1 mol/L of phosphate buffer containing 1 mol/L of sodium chloride and 1% gelatin. A solution of 0.3 ml was used for recovery estimation.

Assay of prostanoids. The three prostanoids were measured by radioimmunoassay with use of specific antisera by the method described by Hirata.[12] Briefly, 0.1 ml of separated sample, radioactive prostaglandins, and antiserum were mixed and incubated 60 minutes at 37° C. Bound-free separation was performed by dextran-coated charcoal solution. After centrifugation, 0.3 ml of supernatant was counted by LSC 30 liquid scintillation counter (Aloka, Tokyo, Japan).

Cross-reactivity of each antisera. Cross-reactivity of each antisera is shown in Tables IA and IB. Since 13,14-dihydro-15-keto $PGF_{2\alpha}$ antiserum had high cross reactivity to 13,14-dihydro-15-keto $PGF_{1\alpha}$, these two prostaglandins were not distinguished. Therefore, the result was described as 13,14-dihydro-15-keto PGF (PGFM).

Coefficiency and recovery rate of radioimmunoassay. The intra-assay coefficiency values for 6-keto $PGF_{1\alpha}$, TXB_2, and PGFM radioimmunoassays were 4.1%, 4.6%, and 3.9%, respectively. The interassay

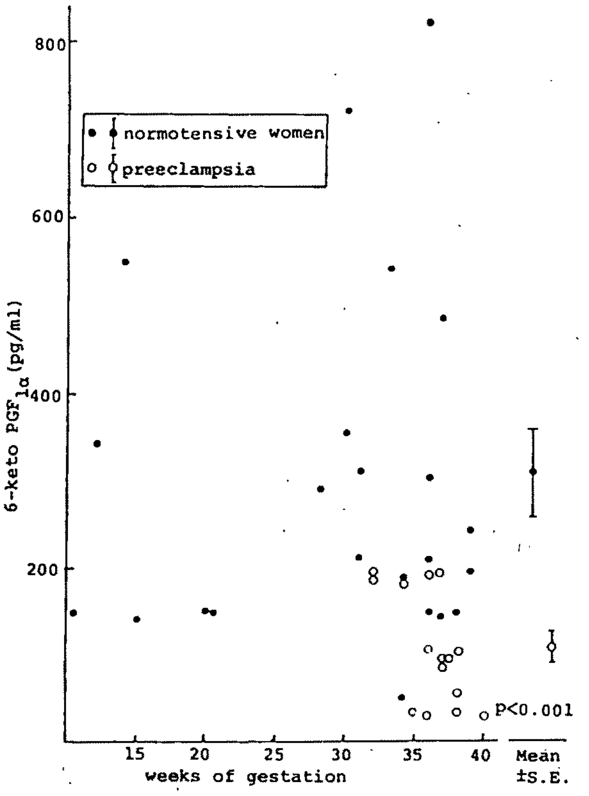

Fig. 1. 6-Keto PGF$_{1\alpha}$ concentration during pregnancy. 6-Keto PGF$_{1\alpha}$ concentrations of the preeclamptic group were significantly lower ($p < 0.001$) than those of the normotensive group.

Table III. 6-Keto PGF$_{1\alpha}$ concentration (pg/ml, mean ± SE) in maternal and umbilical venous plasma in normotensive and preeclamptic patients

Group	*Maternal plasma*	*Umbilical venous plasma*
Normotensive	410.7 ± 51.4*	512.8 ± 73.2*
Preeclamptic	184.2 ± 55.2*	230.9 ± 48.9*

*$p < 0.01$.

coefficiency values for 6-keto PGF$_{1\alpha}$, TXB$_2$, and PGFM radioimmunoassay were 12.8%, 10.0%, and 18.0%, respectively. Recovery rate of each prostanoid was 55.0 ± 3.4% in 6-keto PGF$_{1\alpha}$, 57.2 ± 4.0% in TXB$_2$, and 73.8 ± 7.8% in PGFM, respectively.

Statistics. Statistical analysis was performed by Student's *t* test.

Results

Prostanoid concentrations of normotensive and preeclamptic groups are shown in Table II and Figs. 1 to 3. From 30 to 40 weeks of gestation, 6-keto PGF$_{1\alpha}$ concentrations were 318.6 ± 58.0 pg/ml in the normotensive group and 118.8 ± 16.4 pg/ml in the preeclamptic group (each value is mean ± SE). PGFM values in normotensive and preeclamptic groups were 145.7 ± 20.0 pg/ml and 247.1 ± 48.5 pg/ml, respectively. TXB$_2$ levels were 56.6 ± 7.3 pg/ml in the normotensive group and 119.5 ± 34.0 pg/ml in the preeclamptic group. The 6-keto PGF$_{1\alpha}$ concentration of the preeclamptic group was significantly lower ($p < 0.001$) than that of the normotensive group. However, PGFM and TXB$_2$ levels of the preeclamptic group did not significantly differ from those of the normotensive group.

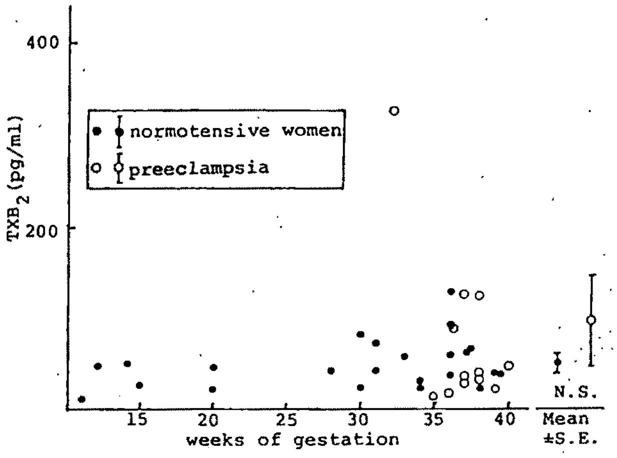

Fig. 2. TXB$_2$ concentration during pregnancy. TXB$_2$ levels of the preeclamptic group did not significantly differ from those of the normotensive group.

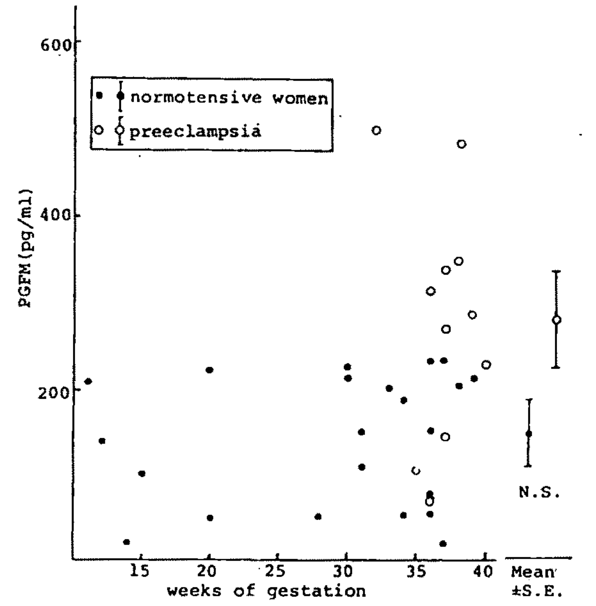

Fig. 3. PGFM concentration during pregnancy. PGFM levels of the preeclamptic group did not significantly differ from those of the normotensive group.

6-Keto PGF$_{1\alpha}$ levels in umbilical venous and maternal plasma at delivery are presented in Table III and Fig. 4. 6-Keto PGF$_{1\alpha}$ levels of normotensive and preeclamptic patients were 410.7 ± 51.4 and 184.2 ± 55.2 pg/ml

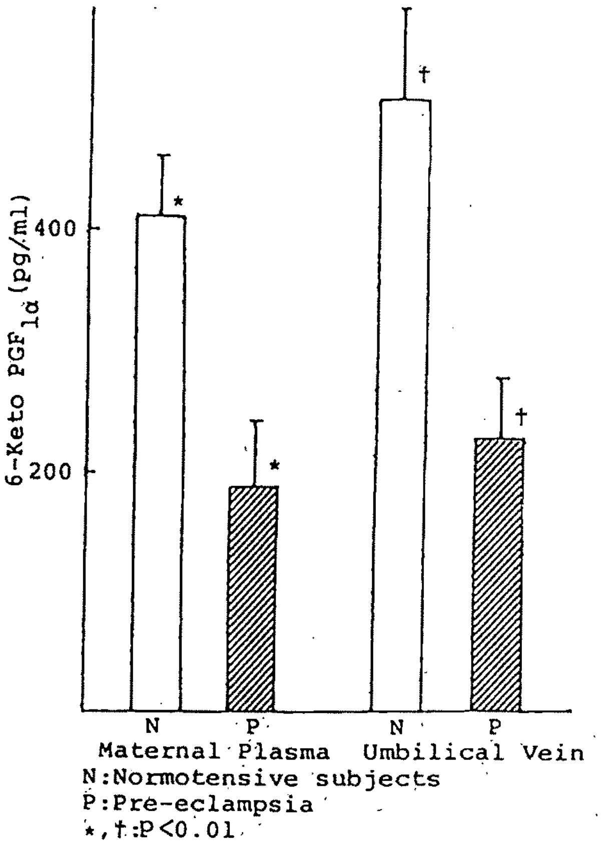

Fig. 4. 6-Keto $PGF_{1\alpha}$ concentration in maternal and umbilical venous plasma. 6-Keto $PGF_{1\alpha}$ levels of the preeclamptic group were significantly lower than those of the normotensive group in both maternal and umbilical venous plasma.

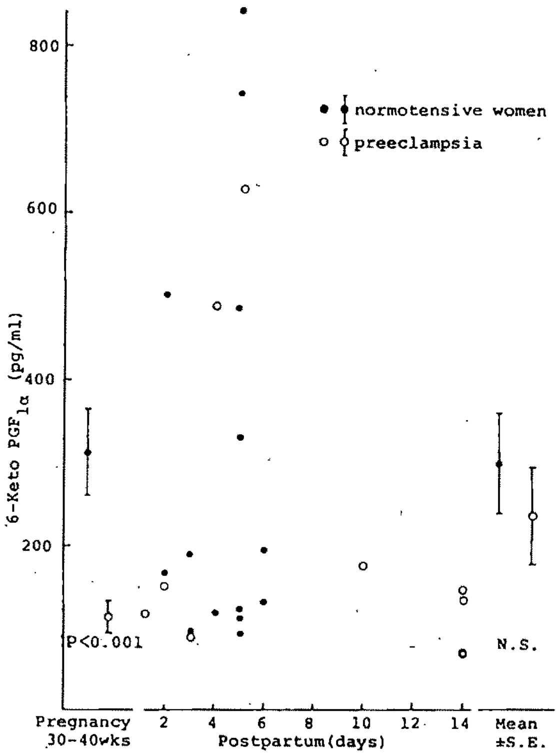

Fig. 5. 6-Keto $PGF_{1\alpha}$ concentration during puerperium. Although 6-keto $PGF_{1\alpha}$ levels of the preeclamptic group before delivery were significantly lower than those of the normotensive group, no significant differences could be observed between normotensive and preeclamptic groups in the postpartum period.

in maternal plasma and 512.8 ± 73.2 and 230.9 ± 48.9 pg/ml in umbilical venous plasma, respectively.

The 6-keto $PGF_{1\alpha}$ level of the preeclamptic group was significantly ($p < 0.01$) lower than that of the normotensive group in both maternal and umbilical venous plasma.

Prostanoid levels in the postpartum period are shown in Table II and Figs. 5 to 7. 6-Keto $PGF_{1\alpha}$ levels in the postpartum period were 298.1 ± 66.9 pg/ml for the normotensive postpartum group and 217.7 ± 62.0 pg/ml for the preeclamptic group. PGFM values were 253.5 ± 21.4 pg/ml for the normotensive postpartum group and 490.0 ± 134.4 pg/ml for the preeclamptic group. TXB_2 concentrations were 127.5 ± 28.4 pg/ml in the normotensive group and 172.7 ± 91.9 pg/ml in the preeclamptic group. Although the 6-keto $PGF_{1\alpha}$ level for the preeclamptic group before labor was significantly lower than that for the normotensive group, no significant difference could be observed between normotensive and preeclamptic groups in the postpartum period. PGFM and TXB_2 values also were not significantly different between normotensive and preeclamptic postpartum groups. The 6-keto $PGF_{1\alpha}$ concentration did not change after delivery. However, PGFM and TXB_2 levels were significantly ($p < 0.05$) elevated after delivery.

Serial measurement of prostanoids in eight patients of the preeclamptic group are presented in Figs. 8 to 10. There was no definite tendency for elevation or depression in the level of each prostanoid before and after labor. Interestingly, however, in six out of eight patients an elevation in 6-keto $PGF_{1\alpha}$ concentration occurred after delivery.

Comment

Prostacyclin and thromboxane A_2, newly discovered unstable prostanoids, have been studied for their powerful pharmacologic effects. There are several reports describing a more potent hypotensive effect for prostacyclin than for other prostanoids. Most of these reports presumed the possibility of prostacyclin's control of blood pressure or maintenance of peripheral circulation.[13-15] Elevated plasma 6-keto $PGF_{1\alpha}$ concentration during pregnancy raises the possibility that vasoactive prostanoids may control blood pressure during pregnancy.[16] In fetal circulation, Remuzzi et al.[3] and Kawano et al.[17] reported a significantly higher production rate of prostacyclin in fetal vessels. The high pros-

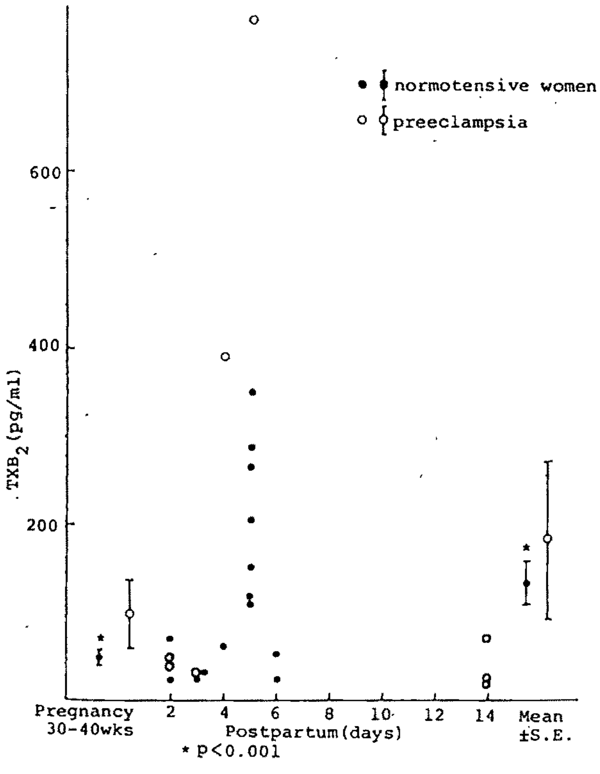

Fig. 6. TXB$_2$ concentration during puerperium. TXB$_2$ levels of the normotensive group were significantly elevated after delivery.

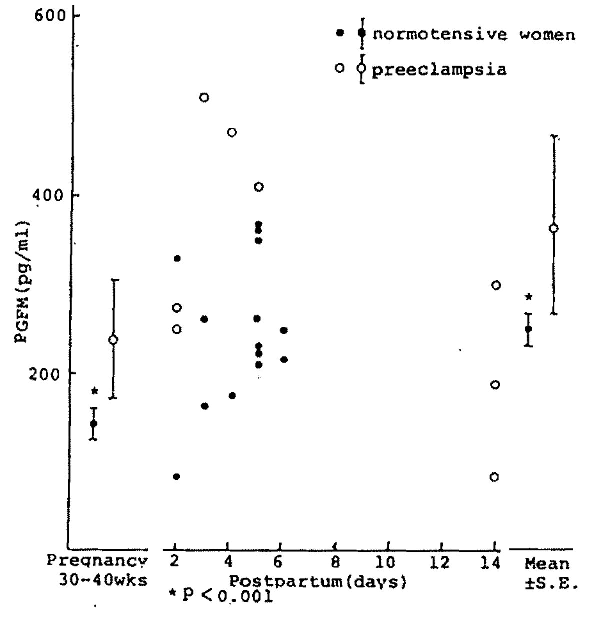

Fig. 7. PGFM concentration during puerperium. PGFM levels of the normotensive group were significantly elevated after delivery.

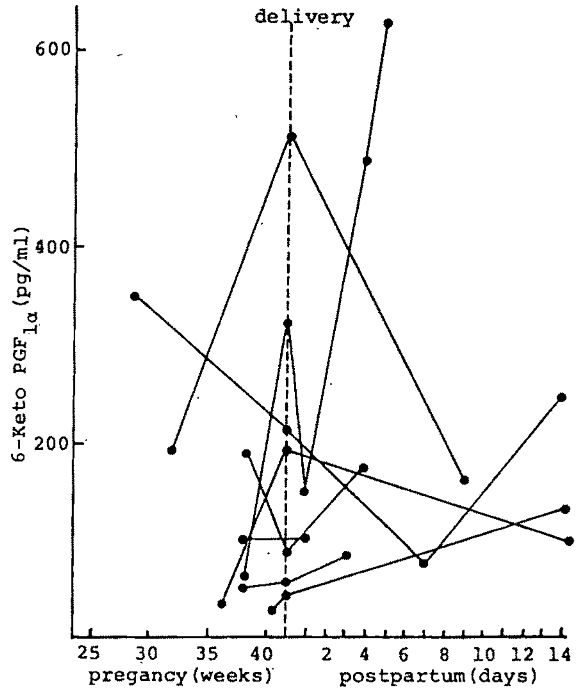

Fig. 8. Serial measurement of 6-keto PGF$_{1\alpha}$ in preeclamptic patients. Note that in six of eight patients, prostanoid level was elevated after delivery.

tacyclin production reported may be related to the maintenance of fetal circulation.[17]

In some pathologic conditions, prostacyclin is considered to have an important role in the pathophysiology of disease. Pace-Asciak et al.[18] reported enhanced formation of prostacyclin in the spontaneous hypertensive rat. Gullner et al.[19] presented evidence of an overproduction of prostacyclin in Bartter's syndrome. A suppressed 6-keto PGF$_{1\alpha}$ level was also noted in women taking oral contraceptives.[20] In preeclampsia, some investigators[4, 5, 21-23] have reported depressed in vitro production of prostacyclin or 6-keto PGF$_{1\alpha}$ in placental and umbilical vessels. Moreover, a reduced quantity of the enzyme complex for prostacyclin synthesis was observed, although the activity of this enzyme complex was the same as in normal subjects.[21] Elevated TXB$_2$ levels and depressed 6-keto PGF$_{1\alpha}$ levels in the amniotic fluid of preeclamptic patients also have been observed.[24]

The levels of prostacyclin or 6-keto PGF$_{1\alpha}$ in the peripheral plasma of preeclamptic patients are still debated.[7-11] The results of this study are different from those of Ylikorkala et al.[10] or Koullapis et al.[11]. One possible explanation for the disagreement is a difference in the purification method. In our previous study we suggested that elevated serum lipids might inhibit antigen-antibody reaction in the radioimmunoassay system.[25] Inhibition of the reaction by lipids was also described by Hensby et al.[26] Another possibility is a difference in criteria in selecting the study group. In this study we dealt only with severe preeclamptic patients. Tsukatani[7] reported that there was no significant difference of plasma 6-keto PGF$_{1\alpha}$ concentration be-

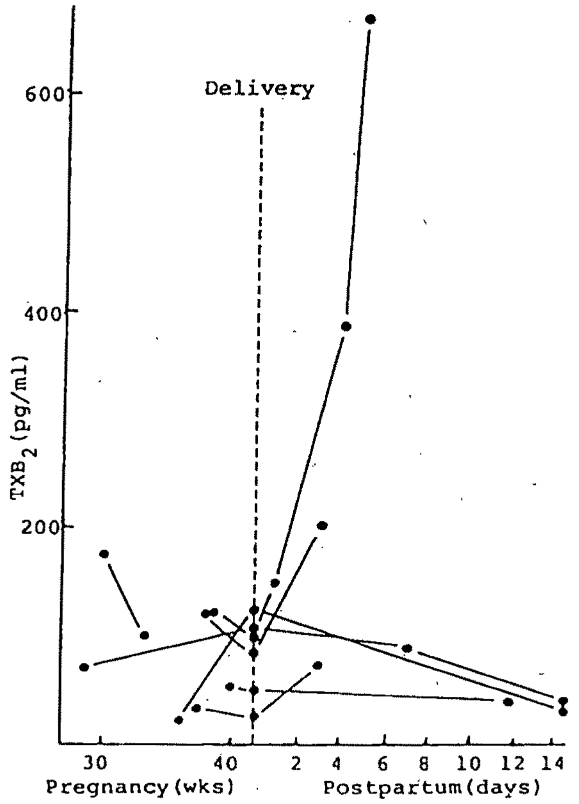

Fig. 9. Serial measurement of TXB_2 in preeclamptic patients. There was no definite tendency of elevation or depression in the level of TXB_2 before and after delivery.

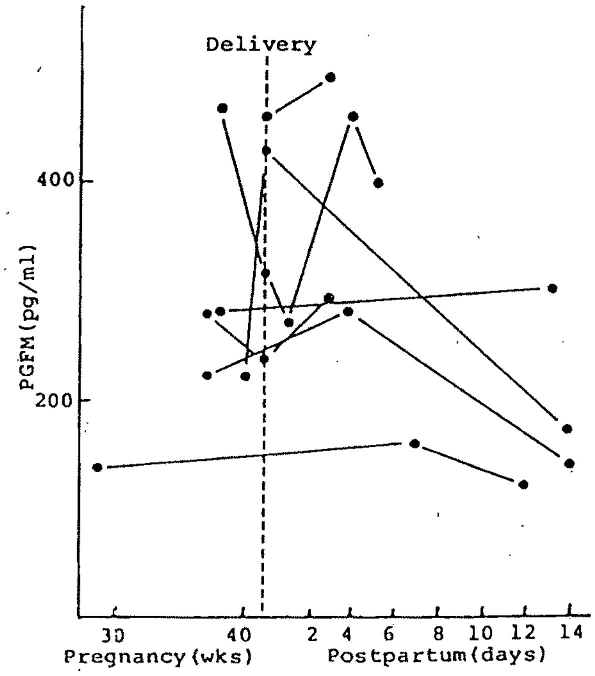

Fig. 10. Serial measurement of PGFM in preeclamptic patients. There was no definite tendency of elevation or depression in the level of PGFM before and after delivery.

tween normotensive and mild preeclamptic pregnant women, whereas 6-keto $PGF_{1\alpha}$ levels in severe preeclampsia were significantly lower than those for both normotensive and mild preeclamptic groups.

In fetal plasma we observed significantly lower 6-keto $PGF_{1\alpha}$ concentrations in the fetuses of the preeclamptic group than in those of the normotensive group at delivery. But even in preeclampsia the fetal 6-keto $PGF_{1\alpha}$ level was higher than the maternal level. Therefore the suppressed plasma 6-keto $PGF_{1\alpha}$ level of the preeclamptic fetus might be due to suppressed prostacyclin production in fetal vessels. Our results may be related to the pathophysiology of intrauterine growth retardation associated with preeclampsia when considering significantly increased fetal vascular resistance in intrauterine growth retarded fetuses as reported by Jouppila et al.[27] It is argued that depressed 6-keto $PGF_{1\alpha}$ levels may be due to low fetal weight in preeclampsia.[28] In our study, fetal weights of the preeclamptic group were somewhat lower than those of the normotensive group. However, there was no significant correlation ($r = 0.030$) between fetal weight and plasma 6-keto $PGF_{1\alpha}$ concentration in both fetal and maternal plasma in normotensive and preeclamptic groups.

In the postpartum period, depressed 6-keto $PGF_{1\alpha}$ levels of the preeclamptic group returned to a normal range within several postpartum days. On the fifth postpartum day there was no significant difference between the preeclamptic and the normotensive group. In six out of eight preeclamptic patients in whom prostanoids were measured serially throughout pregnancy and puerperium, 6-keto $PGF_{1\alpha}$ concentrations were elevated soon after delivery. The clinical preeclamptic symptoms, except for proteinuria, soon disappeared in most of our patients. From our results, it seems that prostacyclin plays an important role in preeclampsia in both mother and fetus.

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Localization of transferrin receptor in the chorionic villi of complete molar pregnancy

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Transferrin receptors have been identified on the villous trophoblast of normal chorionic villi by immunohistochemical techniques. The current study investigated the expression of transferrin receptors on molar chorionic villi by using a monoclonal antibody in immunofluorescence assays. The villous trophoblast of molar chorionic villi was brightly positive for transferrin receptor in the immunofluorescence assay. The presence of transferrin receptors on molar villous trophoblast may influence the immunologic relationship between molar and host tissues. (AM J OBSTET GYNECOL 1985;151:128-9.)

Key words: Transferrin receptor, complete mole

The fetus and placenta express paternally derived genes and therefore are partial allografts in the maternal host. Fetal-maternal immunologic relationships are not clearly understood and have been an area of intense study. Transferrin receptors have been identified on the villous trophoblast of normal chorionic villi by immunohistochemical techniques.[1] The dense concentration of transferrin receptors on the villous trophoblast may serve to protect fetal tissues from immunologic rejection by the maternal host.[1] Transferrin receptors on the villous trophoblast bind maternal transferrin, a large molecule which could mask other adjacent foreign antigenic sites on the trophoblast surface. Furthermore, trophoblast transferrin receptors may deplete the supply of transferrin in the intervillous space and this may impede the proliferation and function of maternal lymphocytes at the maternal-fetal interface.

Complete molar pregnancies generally have a 46,XX karyotype, and molar chromosomes are entirely derived from paternal origin. Therefore, molar tissue is a complete allograft and has the potential to induce a vigorous maternal host immunologic response. Evidence of maternal immunologic reactions to molar pregnancy has been reported, but the immunologic interactions between molar and host tissues are not well understood. The current study was undertaken to determine by immunofluorescence assay whether transferrin receptors are detectable on the chorionic villi of complete molar pregnancy.

Placental tissue was obtained at 13, 16, 20, and 40 weeks' gestation at the time of therapeutic abortion or term delivery, and molar tissue was obtained by suction curettage in four patients with complete hydatidiform mole. The placental and molar tissues were washed to remove blood elements, frozen in liquid nitrogen, embedded in optimum cutting temperature compound (Lab-Tek Products, Naperville, Illinois), and sectioned at 6 μm. One section from each tissue was fixed in methanol and stained with hematoxylin and eosin. The remaining frozen sections were fixed in cold methanol for 10 minutes and stored at −70° C until used for immunofluorescence studies. Two human gestational choriocarcinoma cell lines (JAR and BeWo), which were obtained from Dr. R. A. Pattillo (Medical College of Wisconsin, Milwaukee, Wisconsin), were grown on glass coverslips and processed with the placental and molar tissues.

A monoclonal antibody (66-IG10) directed against the human transferrin receptor was obtained from Dr. M. van de Rijn (Dana Farber Cancer Institute, Boston, Massachusetts).[2]

Fixed sections were incubated briefly in phosphate-buffered saline containing 1% bovine serum albumin (PBS-BSA) and lightly counterstained with Evans blue to quench tissue autofluorescence. The monoclonal antibody was diluted 1 : 100 in PBS-BSA and centrifuged at 15,000 × g for 10 minutes. For each tissue and cell line, one slide was incubated with antibody and another slide was incubated with PBS-BSA for 20 minutes at

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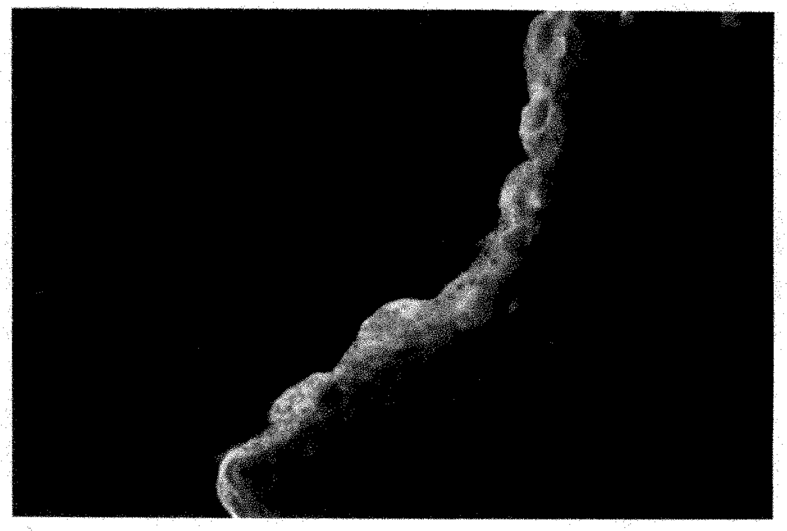

Fig. 1. Photomicrograph of a molar chorionic villus showing expression of transferrin receptor by immunofluorescence assay. While the villous trophoblast is brightly positive for transferrin receptor, cells of the villous stroma show no reactivity with anti–transferrin receptor antibody. (Original magnification, × 1,300.)

room temperature in a humidified chamber, washed in PBS-BSA, incubated with fluorescein isothiocyanate–labeled rabbit antimouse IgG (Cappel Laboratories, Cochranville, Pennsylvania) for an additional 20 minutes and washed in PBS. Slides were then examined with a Zeiss epifluorescence microscope equipped with a mercury lamp and fluorescein filters.

Transferrin receptor was detectable on the villous trophoblast from normal placentas from 13 weeks to term gestation as well as on both choriocarcinoma cell lines. The villous trophoblast of molar chorionic villi was also brightly positive in the immunofluorescence assay for transferrin receptor (Fig. 1). The villous stroma in both the normal placenta and the molar tissues showed no reactivity with anti–transferrin receptor antibody.

Transferrin receptors are expressed by rapidly proliferating cells in vitro and in vivo, and most malignant cells including choriocarcinoma express high levels of this molecule.[3] Many essential reactions in energy metabolism and DNA synthesis are catalyzed by iron-containing enzymes, and transferrin, the major iron-transporting protein, is an obligatory cellular growth factor. When monoclonal antibodies to transferrin receptor block transferrin binding, human tumor cell growth is inhibited in vitro.[3]

Molar pregnancy is well recognized to have a potential for deep myometrial invasion and distant spread. The maternal host's immunologic response to the molar allograft may affect the likelihood of developing postmolar persistent trophoblastic tumor. The presence of transferrin receptors on the molar villous trophoblast could influence the immunologic relationship between molar and host tissues. Transferrin receptors may protect the molar tissue from immunologic rejection by diminishing maternal lymphocyte responsiveness or by masking other trophoblast antigenic sites. However, the expression of transferrin receptor could also accentuate the host's immunologic response because transferrin receptors (possibly in modified form) are apparently target structures for natural killer cells.[4] The immunobiology of complete molar pregnancy may be better understood through further study of the role of trophoblast transferrin receptors on molar-host immunologic interactions.

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Characterization and localization of HLA antigens on hydatidiform mole

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Frozen sections of three specimens of hydatidiform mole were stained with monoclonal antibodies to HLA Class I and Class II antigens by means of an indirect immunoperoxidase technique. Class I (HLA A, B, C) antigens were detected on proliferating extravillous trophoblast and on villous stromal cells but not on quiescent villous trophoblast. Trophoblast Class I antigen was detected with four different antibodies to monomorphic determinants but not with antibodies to the appropriate polymorphic HLA A or B type. Stromal cells were reactive with all Class I antibodies. Class II (HLA DR) antigens were not detected on any molar tissue. The expression of HLA antigens by molar tissue is similar to that of the normal first-trimester human placenta. (AM J OBSTET GYNECOL 1985;151:130-5.)

Key words: HLA antigens, hydatidiform mole, trophoblast, monoclonal antibodies

The normal placenta, hydatidiform mole, and gestational choriocarcinoma share the unusual property of being natural allografts in the sense that they are genetically disparate tissues in intimate contact with a fully immunocompetent host. In most circumstances the natural allograft is successful; pregnancy continues to term and choriocarcinoma, if left untreated, leads to the death of the sufferer.

It is now clear that the major histocompatibility antigens (HLA) of man function not only as the major inducers of, and targets for, artificial tissue allograft rejection but also as essential mediators in normal cellular immunologic reactions. Precise determination of the nature and localization of HLA antigens on placenta and trophoblast tumors is therefore necessary if we are to understand immunologic reactions toward them.

Host recognition of the HLA system of the conceptus clearly occurs during both normal and molar pregnancy, as shown by the presence of paternal-specific HLA antibodies in maternal blood prior to delivery.[1, 2] Several studies have demonstrated the absence of both Class I (HLA A, B, C) and Class II (HLA DR) antigens on the villous trophoblast of the normal placenta.[3-5] In contrast, cells of the villous stroma, which form the central part of the villi, do express class I antigens throughout gestation. Class II antigens are found on specific dendrite-like cells in the villous stroma in third-trimester placentas.[5, 6] Such stromal antigens are separated from maternal tissue by the HLA-deficient villous trophoblast, which is thought to make up a continuous layer around the villi. Recent studies have shown that several populations of nonvillous trophoblast express Class I antigens.[7, 8] Such trophoblast is thought to arise from primitive villous trophoblast during early gestation and comes to lie in direct contact with maternal uterine tissue for the remainder of the pregnancy. Such antigens, however, can only be detected with antibodies to monomorphic or constant determinants of HLA A, B, C, and not with antibodies directed to the polymorphic, or variable, regions of the molecule.

In this paper we report a similar study of hydatidiform mole. This benign placental tumor is characterized by aberrant trophoblast proliferation and hydropic degeneration of the villi. It occurs in up to 1 in 250 pregnancies in some parts of the Far East but only 1 in 2000 in Europe and the continental United States.[9] Two distinct types have been identified within the hydatidiform mole classification.[10-12] Complete mole refers to a tumor of androgenetic origin whose XX chromosome constitution is exclusively paternal and which presents with the classical vesicular or grapelike appearance due to generalized hydropic degeneration and trophoblast proliferation. A fetus is never present. In contrast partial moles are triploid, bearing maternal and paternal genes; hydropic degeneration is partial and trophoblastic proliferation is focal. There is normally evidence of a fetus. Approximately 9% of complete moles develop into some form of persistent trophoblastic disease, usually choriocarcinoma.[9]

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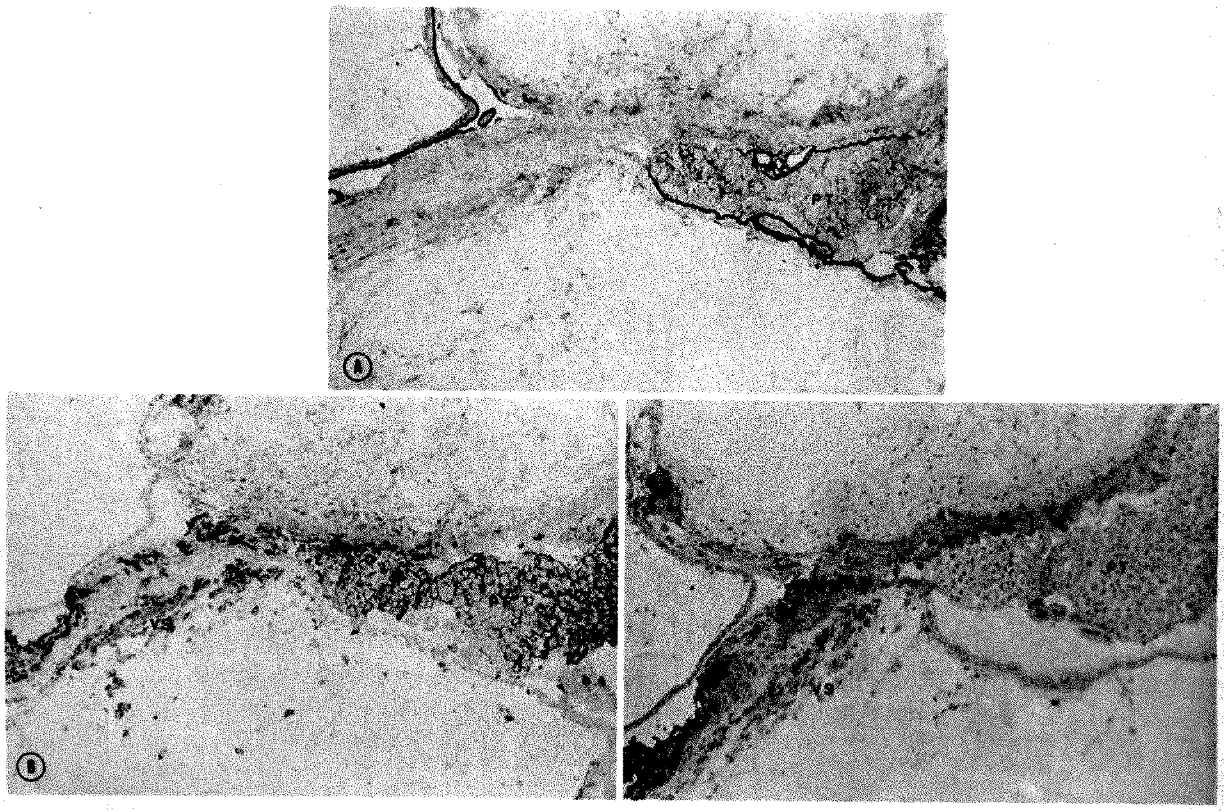

Fig. 1. Serial sections of Mole 1 were stained with monoclonal antibodies with the indirect immunoperoxidase technique. *A*, NDOG 1 monoclonal antibody stains the syncytiotrophoblast plasma membrane and some components within the column of proliferating extravillous trophoblast (*PT*). *B*, W6/32 monoclonal antibody, which detects a monomorphic determinant of Class I (HLA A, B, C) antigens, stains proliferating extravillous trophoblast (*PT*), villous stromal cells (*VS*), and some maternal leukocytes trapped in a fibrinous layer external to the villous trophoblast (*L*). *C*, NFK-1 monoclonal antibody, which detects a monomorphic determinant of Class II (HLA-DR) antigens, stains no molar tissue. Labeling is limited to maternal lymphocytes (*L*) as in *B*. (Nuclei were counterstained with hematoxylin. Original magnification × 120.)

We have collected and studied samples from three hydatidiform moles with the use of a panel of monoclonal antibodies in an indirect immunoperoxidase technique on frozen sections.

Patients and methods

Specimens with the histopathologic diagnosis of hydatidiform mole were taken from three patients. They were classified into complete or partial moles on the basis of the morphologic criteria described by Szulman and Surti[11, 12] after examination of several blocks of tissue from each patient. It has been previously shown that there is an excellent correlation between morphologic and cytogenetic classification of moles.[19]

Mole 1. Molar tissue was evacuated at 16 weeks' menstrual age. The diagnosis was initially suggested by a routine ultrasound scan at 13 weeks and then confirmed ultrasonically and biochemically. Microscopically, all villi showed marked hydatidiform change with gross swelling and central cistern formation. There was moderate trophoblastic proliferation around most villi. There was neither macroscopic nor microscopic evidence of a fetus. Mole 1 was designated as a complete mole.

Mole 2. Molar tissue was evacuated from a patient with uncertain dates after detection by ultrasound. Histologic analysis showed enlarged villi containing an immature mesenchymal stroma. There was moderate trophoblastic proliferation and moderate anaplasia throughout the specimen. There was neither macroscopic nor microscopic evidence of a fetus. Mole 2 was designated a complete mole. Villi were generally smaller and the stroma more dense than those of Mole 1, suggesting the specimen was of an earlier gestation.

Mole 3. Molar products were observed after the suction termination in a teenager of a pregnancy previously considered normal. There was marked variability in the size of the villi, including some with normal mesenchymal stroma. Nucleated erythrocytes were detected in fetal capillaries. Trophoblastic cysts or "fjords"

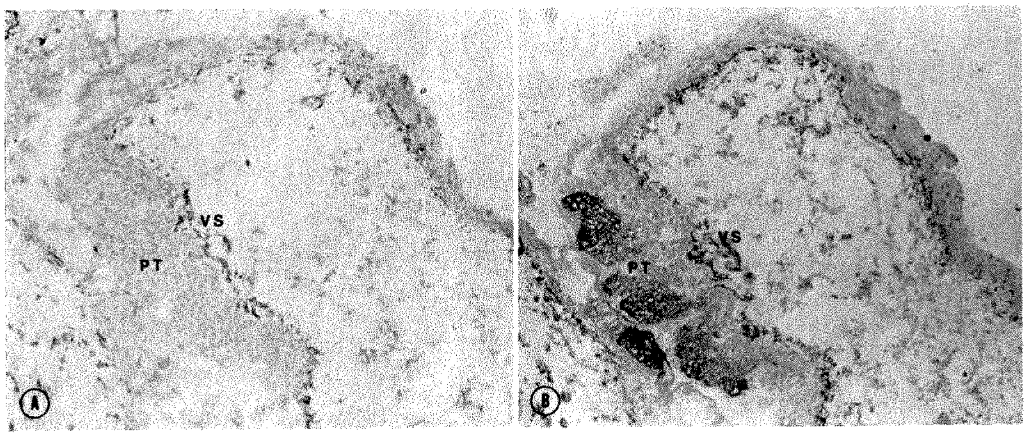

Fig. 2. Serial sections of Mole 2 were stained with monoclonal antibodies with the indirect immunoperoxidase technique. *A*, The antibody ME1, which specifically detects HLA B7, B22, B27, stains only cells of the villous stroma (*VS*), while (*B*) W6/32 stains, in addition, areas of proliferating trophoblast (*PT*). (Nuclei were counterstained with hematoxylin. Original magnification × 120.)

Table I. Monoclonal antibody preparations

Monoclonal antibody	*Specificity*	*Reference*	*Preparation*
NDOG1	Villous syncytiotrophoblast plasma membrane, some nonvillous trophoblast, unreactive with most adult tissues including liver, kidney, heart, brain, colon, pancreas, and pregnant uterus	7	Tissue culture supernatant
NFK-1	Class II (HLA DR) antigens,* monomorphic†	15	Ascites
W6/32	Class I (HLA A, B, C) antigens, monomorphic	16	Ascites
BB 7.7	Class I (HLA A, B, C,) antigens, monomorphic	16	Immunoglobulin
BB 7.8	Class I (HLA A, B, C,) antigens, monomorphic	16	Immunoglobulin
BBM1	β_2-Microglobulin	16	Immunoglobulin
MA 2.1	Class I HLA A2 and B17	17	Ascites
ME 1	Class I HLA B7, B22 and B27	18	Ascites

*The reactivity of this antibody with the DC and SB Class II locus products has not yet been determined.

†The term monomorphic means that the determinant recognized is common to all HLA antigens of this class. In contrast, antibodies MA 2.1 and ME 1 react only with certain HLA A and B specificities and are therefore deemed to react with a "polymorphic" antigenic determinant.

as described by Szulman and Surti were observed. There was only slight trophoblastic proliferation. Mole 3 was designated as a partial mole.

Small pieces of tissue were taken and frozen in liquid nitrogen. Sections (5 to 8 μm) were then cut in a cryostat. The slides were air dried for 2 hours, fixed in acetone at room temperature for 10 minutes, then washed in 0.15 mol/L of sodium chloride and 0.05 mol/L of Tris hydrochloride, pH 7.6. Sections were incubated with monoclonal antibody (50 μl) at a predetermined saturating concentration for 40 minutes at room temperature. They were then washed twice in Tris–saline solution and incubated similarly with 50 μl of peroxidase-conjugated rabbit antimouse immunoglobulin (Dako) at a 1:50 dilution in Tris–saline solution and 10% normal human serum. Slides were again washed and incubated with 0.6 mg/ml of diaminobenzidine-tetrahydrochloride and 0.007% hydrogen peroxide in Tris–saline solution. The reaction was stopped after 8 minutes by immersion in excess water; the nuclei were counterstained with hematoxylin, and sections were then dehydrated and mounted in DPX. Photomicrographs were taken on a Zeiss photomicroscope III. Figs. 1 and 2 were taken through a blue filter.

The monoclonal antibody preparations used in this study are described in Table I. An irrelevant monoclonal antibody was used as a negative control in all experiments.

Results

Large areas of trophoblastic proliferation were evident in all three hydatidiform moles examined. One such area is illustrated in Fig. 1. In the normal first-trimester placenta, the NDOG1 monoclonal antibody is known to react specifically with the syncytiotrophoblast plasma membrane and with some extracellular

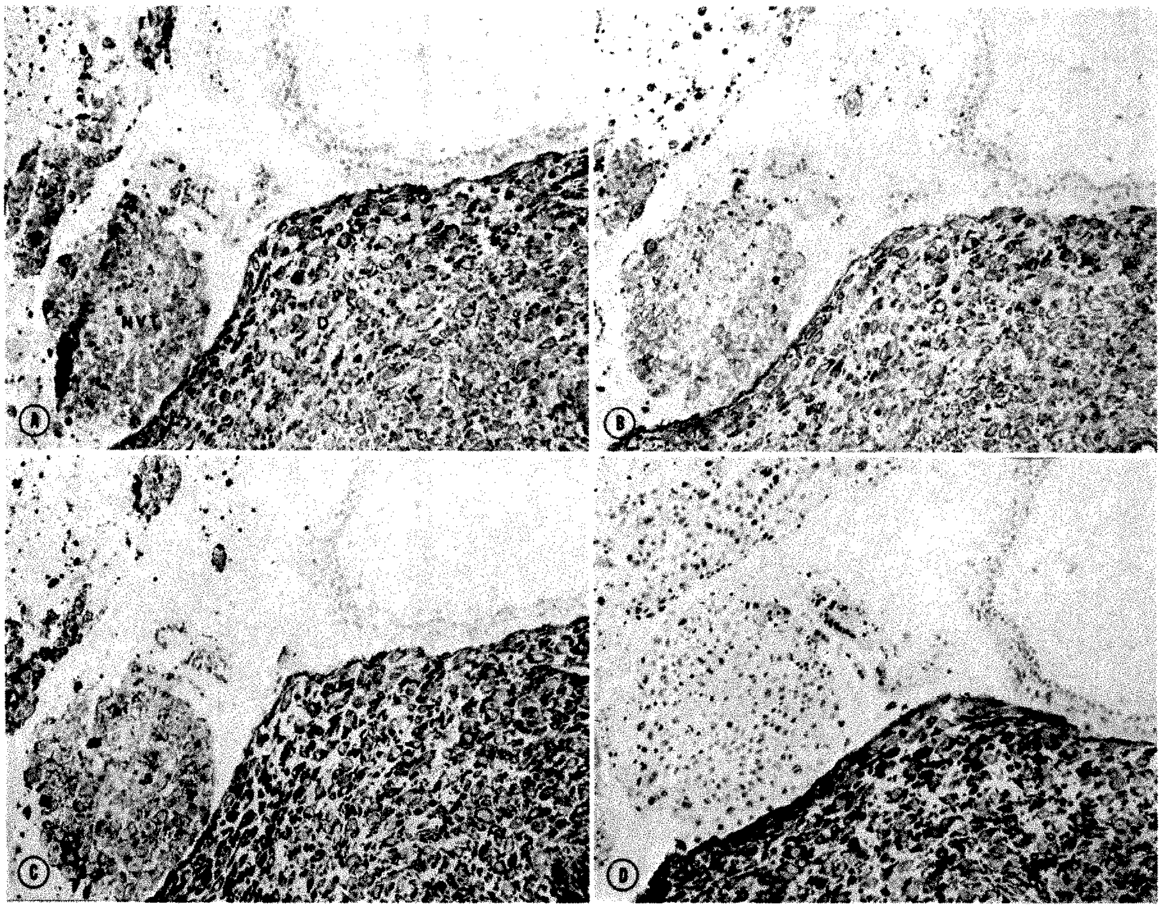

Fig. 3. Serial sections of Mole 3 were stained with three different monoclonal antibodies specific for monomorphic determinants of Class I antigens. These were (*A*) BBM 1, (*B*) BB7.7, and (*C*) BB7.8. All three antibodies labeled an island of nonvillous trophoblast (*NVT*) and an area of maternal decidua (*D*). The ME 1 antibody stains maternal decidual tissue only (*D*). (Nuclei were counterstained with hematoxylin. Original magnification × 120.)

material associated with proliferating cytotrophoblast cell columns.[7] It does not react with most other normal human tissues, including maternal decidual cells. Similarly, in the mole (Fig. 1, *A*), NDOG1 defines an area in which the continuity of the densely labeled syncytiotrophoblast plasma membrane has been broken by the outward proliferation of cytotrophoblast presenting as a column of cells with associated NDOG1 staining proceeding from the site of proliferation. Staining for Class I (HLA A, B, C) antigens with the W6/32 monoclonal antibody (Fig. 1, *B*) shows that this proliferating nonvillous trophoblast population and villous stromal cells both express the Class I antigen while the quiescent areas of villous trophoblast are negative. A similar study of several areas from all three hydatidiform moles showed the same localization of Class I HLA antigens. It was noted, however, that staining of the nonvillous trophoblast was not uniform. Antigen expression was more common on the cellular cytotrophoblast than on the syncytial giant cells and in each mole some areas of proliferating cytotrophoblast were found that did not express the Class I antigen. No staining of any molar tissue was detected with the NFK-1 antibody to Class II (HLA DR) antigens (Fig. 1, *C*), positive reactions being limited to some leukocytes present in the intervillous space.

Molar tissue was next examined with two antibodies specific for polymorphic determinants of HLA A, B, C. These were MA 2.1, specifically reactive with HLA A2 and B17, and ME 1, specific for HLA B7, B22, and B27. In each case the reactivity of the conceptus was assessed by immunohistologic examination of the molar villous stroma with each antibody. Similar examination of maternal leukocytes in the intervillous space or decidua gave the maternal reactivity. The results are given in Table II. The alleles A2, B17, B7, B22, and B27 occur in the Caucasian population with gene frequencies of 25%, 6%, 11%, 13%, and 4%, respectively.[21] This means that MA 2.1 and ME 1 should each theoretically be reactive with approximately 50% of individuals. The results of Table II are consistent with this prediction.

In Moles 2 and 3, it was found that despite clear

Table II. Typing of mother and conceptus, in terms of reactivity with polymorphic HLA antibodies

Mole	*MA 2.1*	*ME 1*
Mole 1		
Mother	+	–
Conceptus	–	–
Mole 2		
Mother	+	+
Conceptus	–	+
Mole 3		
Mother	–	+
Conceptus	+	–

Typing was performed by the indirect immunoperoxidase technique on frozen sections. Maternal reactivity was assessed by staining of decidual tissue or lymphocytes in the intervillous space, while staining of villous stromal cells gave the reactivity of the conceptus.

staining of villous stromal tissue with one of these antibodies, the same antibody on the same sections never labeled proliferating trophoblast. This is illustrated in Fig. 2, *A*. By contrast a serial section with W6/32, detecting monomorphic determinants of Class I (HLA A, B, C) antigens, shows clear staining of this same region of proliferating trophoblast (Fig. 2, *B*). It has been suggested that this discrepancy between staining with W6/32 antibody and antibodies to polymorphic determinants is due to some unique property of the W6/32 monoclonal antibody. We have therefore examined molar tissue with other antibodies directed to monomorphic determinants of Class I (HLA A, B, C) antigens. Fig. 3 shows an area of Mole 3 made up of maternal decidual tissue (*D*), an island of nonvillous trophoblast (*NVT*), and a portion of hydropic villus. The antibody BBM 1, which is specific for β_2-microglobulin, was seen to react with both maternal decidua and nonvillous trophoblast (Fig. 3, *A*). Similar results were obtained with antibodies BB 7.7 (Fig. 3, *B*) and BB 7.8 (Fig. 3, *C*) which react with a monomorphic determinant of Class I antigens distinct from that defined by W6/32.[16] The pattern of staining with the polymorphic ME 1 antibody on this tissue was markedly different. This antibody reacted only with maternal tissue in these sections and served to confirm the histologic distinction between decidua and trophoblast (Fig. 3, *D*).

Comment

One previous study reported that the villous trophoblast of hydatidiform mole lacks Class I (HLA A, B, C) antigens, but such antigens are detectable on villous stromal cells.[13] Our data are compatible with these studies but extend them to an analysis of HLA antigens on the proliferating extravillous trophoblast of hydatidiform mole.

It is apparent that the expression of HLA antigens on molar tissue is similar to that of normal first-trimester placenta, in terms of both its nature and its localization. Thus shortly after implantation in normal pregnancy the villous cytotrophoblast proliferates, bursting through the syncytiotrophoblast membrane and forming cytotrophoblast cell columns which invade the maternal decidua and produce the cytotrophoblast shell around the conceptus. This extravillous trophoblast bears an obvious histologic relationship to the proliferating trophoblast of hydatidiform mole. Furthermore both populations express Class I but not Class II HLA antigens[7] and again, in both cases, the Class I antigens are not detectable with antibodies to polymorphic determinants of HLA even when such antibodies are clearly observed to be bound to the fetal villous stromal cells.[8]

The detection of Class I antigens on nonvillous trophoblast with antibodies to monomorphic but not polymorphic determinants may be due to a number of causes which have been discussed in detail elsewhere.[8] Briefly, the discrepancy may arise because trophoblast Class I antigens are biochemically distinct from Class I antigen that have been defined in other tissues. Alternatively it may arise from an antigen dosage effect. Thus a single polymorphic determinant accounts for only 12% to 15% of the total HLA A, B, C antigens as detected with W6/32 antibody on lymphocytes of a heterozygous individual.[9] It may therefore be that antibodies to monomorphic determinants, which react with the whole Class I population, can detect the antigen but the polymorphic antibodies react with so small a subset of the total antigen population as to move below the level of detection with this technique. It is of note that complete molar tissue has a double paternal genotype and so might be expected to express polymorphic determinants at 25% to 30% of the total, that is, the equivalent of a homozygous individual. While it is clearly desirable to extend the study to additional moles and to include cytogenetic data, definite answers to these questions regarding trophoblast HLA will only come through molecular analysis at the biochemical level. This requires the preparation of purified nonvillous trophoblast.

It is known that a portion of primigravid women develop antibodies to maternal Class I antigens during the course of either normal or molar pregnancy. In normal pregnancy such antibodies may arise as a result of "leakage" of fetal lymphocytes into the maternal bloodstream. In complete moles there is no fetus or fetal circulation and so immunization must arise though the molar tissue itself. Yamashita et al.[14] have shown molar tissue to specifically absorb antipaternal HLA antibody. If further study demonstrates that trophoblast Class I antigens are unusual and do not express polymorphic determinants, then the most likely route of immunization might be through the villous stromal cells, brought into contact with the maternal immune system through discontinuity in the villous trophoblast layer. Such discontinuity may occur in the region of the anchoring villi which are responsible for the attachment of the villous placenta to the basal plate.[20]

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Characterization and comparison of two forms of human chorionic gonadotropin from hydatidiform moles with low and high immunoreactivity

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Two glycoproteins (LM-hCG and HM-hCG) with gonadotropic activity were purified from the chorionic tissue of patients with hydatidiform mole with low and high urinary human chorionic gonadotropin levels and were compared to each other. The immunologic, biologic, and physicochemical properties of the two preparations were very similar. Also, the quantities of LM-hCG and HM-hCG recovered from molar tissue from the two types of patients were similar. However, the molar tissue from patients who excreted high levels of human chorionic gonadotropin immunoreactivity also contained other molecular forms with apparent human chorionic gonadotropin immunoreactivity tissues from patients who excreted low levels did not. These latter molecular forms may account for the differences in the levels of human chorionic gonadotropin immunoreactivity excreted by the two types of patients. (Am J Obstet Gynecol 1985;151:136-41.)

Key words: hCG, hydatidiform mole, immunoreactivity, bioactivity

Patients with hydatidiform mole generally have higher urinary human chorionic gonadotropin (hCG) levels than normal pregnant women. This phenomenon is a useful indicator in the diagnosis of this disorder. However, the observation that a small proportion of patients with substantial amounts of molar tissue having low levels of urinary hCG immunoreactivity points to the need for more thorough physicochemical characterization of the hCG produced by this tissue.

In this report, the preparation and characterization of hCG from molar chorionic tissue of patients excreting low and high levels of hCG immunoreactivity are described. The physicochemical properties are compared with each other as well as with those of hCG purified from urine of normal pregnancy.

Material and methods

Molar chorionic tissue was obtained from four patients with low levels of hCG in the urine (2000 to 50,000 IU/L, by the urinary chorionic gonadotropin titration kit and the hemagglutination inhibition reaction, Kyorin Pharmaceutical Co., Tokyo, Japan) and five patients with high levels of hCG (more than 300,000 IU/L). Molar tissue obtained by dilatation and evacuation was homogenized in cold acetone. After washing with cold acetone several times, the tissue was filtered and air-dried. The acetone powder thus obtained was stored at 4° C until use. A part of each tissue was used for pathologic examination and all were histologically diagnosed as moles. One of the four patients with low immunoreactivity and one of the five patients with high immunoreactivity were diagnosed as having invasive mole because of increasing hCG levels during clinical observation after evacuation. After the diagnosis, they received three or four 5-day courses of 20 mg of methotrexate per day intramuscularly. They are healthy with no demonstrable metastasis 2 years after treatment. hCG and hCG-β radioimmunoassay kits were purchased from the Commissariat a l'Energie Atomique (France) via Green Cross Co. (Tokyo, Japan). The sensitivities of these kits are 0.36 ng/ml of hCG and 0.22 ng/ml of hCG-β, respectively. The percentages of cross-reactivity of the hCG kit are: hCG-α, 3.6%; hCG-β, 1.3%; luteinizing hormone, 2.6%; follicle-stimulating hormone, 1.01%; and thyrotropin, 0% and thyrotropin, 0%.

Bioassay of total gonadotropic activity. The gonadotropic activity was determined by the modified Ascheim-Zondek test,[1] which consists of measuring the weight response of both the uterus and the ovaries of immature female mice.

Immunoreactivity of hCG and hCG-β. Immunoreactivity was determined with the use of the commercial radioimmunoassay kits described above according to the manufacturer's instructions.

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Molar tissue
↓
Acetone powder
↓ Extraction with 0.01M PBS (pH 7.4)
Stirring overnight at 4° C
Centrifugation by 10,000 × g for 10 min
Supernatant
↓ 70% Saturation by ammonium sulfate
Centrifugation by 10,000 × g for 10 min
Precipitate
↓
Sephadex G-150
↓
DEAE Sephadex A-50
↓
Con A Sepharose 4B
↓
Sephadex G-150
↓
Purified molar hCG

Fig. 1. Flow diagram for purification of molar hCG.

Analytic disc electrophoresis. Polyacrylamide gel electrophoresis was conducted on the purified hCG as well as on all other fractions obtained during preparation. The polyacrylamide gel electrophoresis technique used was the method of Ishiguro et al.[2]

Chemical analysis of hCG. The amino acid compositions of final products were determined by hydrolysis with 6N hydrochloric acid at 110° C for 24 hours in a vacuum and analysis of the hydrolysate with a JEOL 6AH amino acid analyzer. Sugars were analyzed by the phenol–sulfuric acid method for neutral sugars,[3] by the use of the amino acid analyzer for hexosamines after hydrolysis with 4N hydrochloric acid at 105° C for 4 hours, and by the thiobarbituric acid method for sialic acid.[4]

Extraction and fractionation of molar tissue. The methods for purification of molar hCG are outlined in Fig. 1. After 35 gm of acetone powder of molar chorionic tissue was ground with 50 ml of acetone with a mortar and pestle, 500 ml of 0.01 mol/L of phosphate-buffered saline solution (pH 7.4) was added and the pH of the suspension was adjusted to 7.4 with potassium hydroxide. Extraction was performed with the use of mild stirring overnight in 0.01 mol/L of phosphate-buffered saline solution (pH 7.4) containing 5000 units of aprotinin as a protease inhibitor. The suspension was centrifuged at 10,000 × g for 10 minutes and the residue was again extracted with 300 ml of phosphate-buffered saline solution plus aprotinin as described above. The combined supernatants were brought to 70% saturation with solid ammonium sulfate. After the suspension stood overnight at 4° C, the precipitates were collected by centrifugation at 10,000 × g for 10 minutes and dialyzed against 0.05 mol/L of Tris–hydrochloric acid buffer containing 0.15 mol/L of sodium chloride (pH 7.4). These preparations were then chromatographed using the procedure indicated in the legends to Figs. 2 to 5.

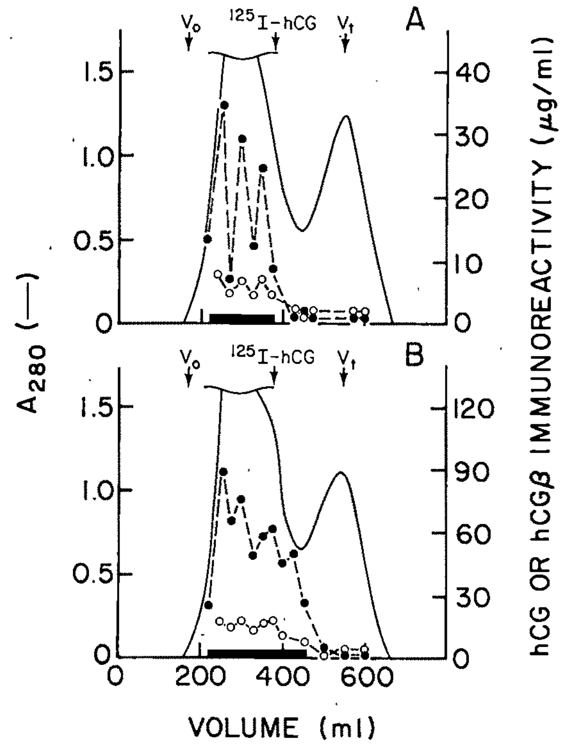

Fig. 2. Gel filtrations of LM-70 *(A)* and HM-70 *(B)* on Sephadex G-150. At first, 0.82 gm of LM-70 and 0.96 gm of HM-70 were passed through a column (2.6 by 90 cm) of Sephadex G-150. The columns were eluted with 0.01 mol/L of ammonium bicarbonate buffer, pH 6.6. The void volume *(V_o)*, total volume *(V_t)*, and elution volume *(V_e)* of urinary hCG of normal pregnancy are indicated by the *arrows*. The fractions designated by *black bars* were pooled (*A:* fraction 1-L and *B:* 1-H). Immunoreactivities of hCG (●---●) and hCG-β (○---○) in 1 ml of buffer.

Results

Purification of LM-hCG. From 35 gm of acetone powder of chorionic tissue of mole with low hCG immunoreactivity, 1.8 gm of protein was extracted with 0.01 mol/L of phosphate-buffered saline solution at pH 7.4 (extract LM). After ammonium sulfate fractionation, the protein fraction obtained at 70% saturation (LM-70) contained 0.82 gm, a recovery of 46%. This LM-70 contained 95% of the gonadotropic activity. The elution profile on Sephadex G-150 is shown in Fig. 2, *A*. Three peaks with hCG and hCG-β immunoreactivity (fraction 1-L) were indicated in molecular weight fractions the same as or larger than those of urinary hCG of normal pregnancy. The elution profile of fraction 1-L on diethylaminoethyl cellulose Sephadex A-50 is shown in Fig. 3, *A*. A total of 88% of immunoreactivity was recovered in the eluate between 0.1 and 0.2 mol/L of sodium chloride (fraction 2-L). Biologic activity was also present in the same eluates. Fraction 2-L, hav-

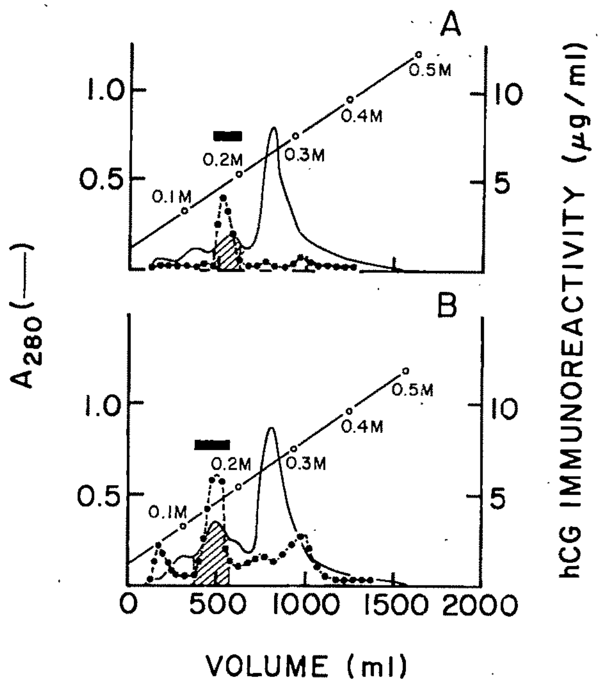

Fig. 3. Chromatographies of fraction 1-L *(A)* and 1-H *(B)* on diethylaminoethyl cellulose Sephadex A-50. The columns (2.6 by 90 cm) were equilibrated with 0.05 mol/L of Tris-phosphate buffer, pH 8.7, and eluted by a linear gradient to 0.5 mol/L of sodium chloride and 0.05 mol/L of Tris-phosphate buffer. The *shaded areas* show the biologically active fractions. The fractions designated by *black bars* were pooled (*A:* fraction 2-L and *B:* fraction 2-H). Immunoreactivities of hCG (●---●) in 1 ml of buffer.

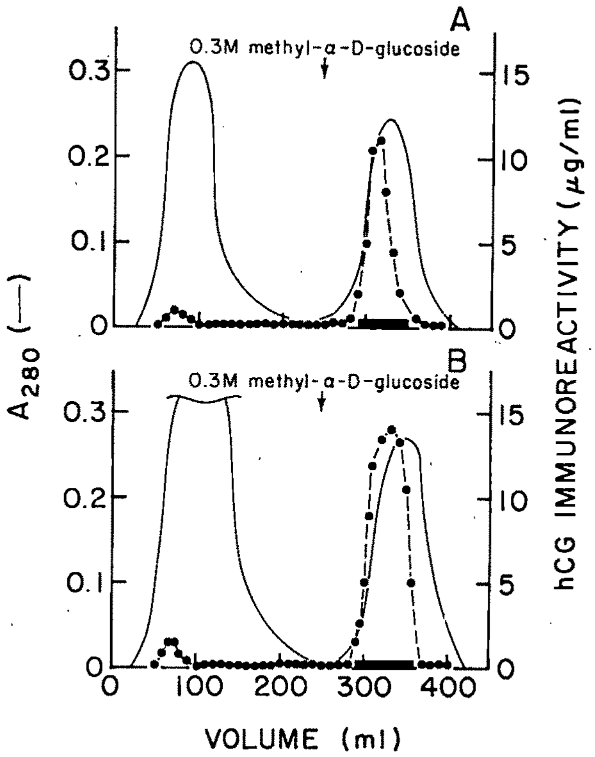

Fig. 4. Affinity chromatography of fraction 2-L *(A)* and 2-H *(B)* on concanavalin A–Sepharose 4B. The columns (1.5 by 50 cm) were equilibrated with 0.05 mol/L of Tris–hydrochloric acid buffer containing 1% butanol and 0.15 mol/L of sodium chloride, pH 7.4, and fractions 2-L and 2-H which had been dialyzed against the same buffer were applied to the columns. After the nonadsorbed protein fractions were washed with the same buffer, the adsorbed glycoprotein fractions were eluted with the same buffer, pH 7.4, containing 0.3 mol/L of α-methyl-D-glucoside. The buffer changes were made at the *arrows*. The fractions designated by *black bars* were pooled (*A:* fraction 3-L and *B:* fraction 3-H). Immunoreactivity of hCG (●---●) in 1 ml of buffer.

ing both immunologic and biologic activity, was subjected to further purification on concanavalin A coupled with Sepharose 4B. As shown in Fig. 4, *A*, very little (3%) immunoreactivity was present in the unadsorbed fraction with 97% of immunoreactivity recovered in the adsorbed fraction (fraction 3-L). Finally, this fraction 3-L was subjected to gel filtration on Sephadex G-150, and a symmetric peak was obtained as shown in Fig. 5, *A*. This fraction (fraction 4-L) having both immunologic and biologic activity was subjected to disc gel electrophoresis. Since only one band was observed, fraction 4-L was considered to be pure LM-hCG.

Purification of HM-hCG. From 30 gm of acetone powder of chorionic tissue of mole with high hCG immunoreactivity, 2.2 gm of protein was extracted (extract HM). Of this, 0.96 gm or 44% was obtained at 70% saturation (HM-70). Also, 90% of gonadotropic biologic activity was recovered in this HM-70. Gel filtration of HM-70 (Fig. 2, *B*) on Sephadex G-150 showed a broader region of immunologic activity than did LM-70 (Fig. 2, *A*). The fractions containing the peaks of immunologically active material (fraction 1-H) were pooled. The elution profile of this fraction 1-H on diethylaminoethyl cellulose Sephadex A-50 is shown in Fig. 3, *B*. As indicated, immunologic activity was present in the eluates at concentrations between 0 and 0.1, 0.1 and 0.2, and 0.2 and 0.3 mol/L of sodium chloride. Biologic activity was found only in the eluate between 0.1 and 0.2 mol/L of sodium chloride (fraction 2-H). The elution profile of this fraction 2-H, having both immunologic and biologic activities, on concanavalin A–Sepharose 4B is shown in Fig. 4, *B*. It indicates that almost 95% of the total immunoreactivity was present in the adsorbed fraction (fraction 3-H). Finally, fraction 3-H was subjected to filtration on Sephadex G-150. Since fraction 4-H represented only a single band on disc electrophoresis, it was considered to be pure HM-hCG.

Characterization of LM-hCG and HM-hCG. The purifications of LM-hCG and HM-hCG from acetone powder are summarized in Table I. The biologic activities of purified LM-hCG and HM-hCG increased about eightyfold and 100-fold with recoveries of 42% and 45%, respectively. Their immunologic activities increased 120-fold and 35-fold with recoveries of 61% and 15%, respectively.

Amino acid composition. The amino acid compositions of LM-hCG and HM-hCG are shown in Table II along with the composition of urinary hCG of normal pregnancy for comparison. LM-hCG and HM-hCG have practically identical compositions; both of them appeared to have a high proportion of acidic amino acids, Glu and Asp, but low Pro content compared to that of urinary hCG of normal pregnancy.

Carbohydrate composition. The carbohydrate compositions of LM-hCG and HM-hCG are also shown in Table II along with that of urinary hCG of normal pregnancy. LM-hCG contained 12.0% total hexose, 4.2% amino sugar, and 0.3% sialic acid. HM-hCG contained 12.7% total hexose, 3.5% amino sugar, and 0.5% sialic acid. The values for hexosamine and sialic acid are considerably lower than those found for urinary hCG of normal pregnancy.

Comment

The present investigation was directed toward the isolation of hCG from chorionic tissue of mole which produced hCG of low immunoreactivity. Very few studies have been carried out on hCG from molar chorionic tissue. Also, mole has not been classified according to the amount of hCG secreted. No previous report has described the properties of molar hCG with low immunoreactivity. In the present study a form of hCG extracted from moles of patients with low immunoreactivity in the urine was characterized and its properties compared with those of hCG extracted from moles of patients with high immunoreactivity in the urine.

LM-hCG was purified from a phosphate-buffered saline solution extract of acetone powder derived from moles of patients with low levels of hCG immunoreactivity in the urine. Approximately 95% of the total biologic activity was recovered in LM-70 of the ammonium sulfate fractionation. Similar results were obtained for HM-hCG purified from moles of patients who excrete levels of hCG immunoreactivity in which 90% of the biologic activity was recovered. Although the first gel filtration of HM-70 seemed to have a broader region of immunoreactivity than LM-70, this might have been simply due to tailing of hCG immunoreactivity.

Ion-exchange chromatography of LM-hCG on diethylaminoethyl cellulose Sephadex A-50 revealed immunoreactivity only in fractions eluted between 0.1 and 0.2 mol/L of sodium chloride. In contrast, during the preparation of HM-hCG, immunoreactivity was present in the eluates at concentrations between 0 and 0.1, 0.1 and 0.2, and 0.2 and 0.3 mol/L of sodium chloride. These results indicate that the hCG present in fraction 1-L is more homogeneous with respect to electric charge than that of fraction 1-H, although the two cannot be differentiated from each other with respect to molecular size. As shown in Table I, total biologic activity of extract LM is very similar to that of extract HM. The biologic activity of LM-hCG is also practically the same as that of HM-hCG. On the other hand, total immunologic activity of LM-70 is considerably less than that of HM-70. In fact, there are several peaks of hCG immunoreactivity which have biologic activity in the ion-exchange chromatography of fraction 1-H.

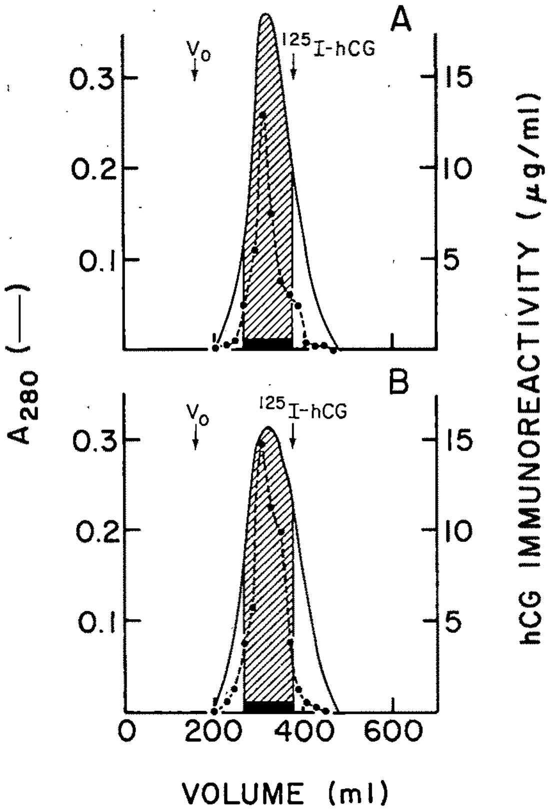

Fig. 5. Gel filtrations of fractions 3-L *(A)* and 3-H *(B)* on Sephadex G-150. Fractions 3-L and 3-H were finally passed through columns (2.6 by 90 cm) of Sephadex G-150. The columns were eluted with 0.01 mol/L of ammonium bicarbonate buffer, pH 6.6. The V_0 and V_t of urinary hCG of normal pregnancy are indicated by the *arrows*. The *shaded areas* show the biologically active fractions. The fractions designated by *black bars* were pooled (*A:* fraction 4-L and *B:* fraction 4-H), dialyzed and lyophilized as purified LM-hCG and HM-hCG. Immunoreactivity of hCG (●---●) in 1 ml of buffer.

The amino acid composition of LM-hCG is practically the same as that of HM-hCG. Both are high in the acidic amino acids, Glu and Asp, but low in Met and half Cys. The characteristically high Pro content of urinary hCG of normal pregnancy was not found in LM-hCG and HM-hCG. The amino acid composition of LM-hCG and

Table I. Summary of yields and potencies

	Total protein (mg)	*Biologic activity (IU/mg)*	*Activity recovery (%)*	*Immunologic activity (IU/mg)*	*Activity recovery (%)*
Extract LM	1800	20	100	45	100
LM-70	820	42	95	84	85
Fraction 2-L	53	510	75	1100	72
Fraction 4-L	9.2	1650	42	5400	61
Extract HM	2200	15	100	150	100
HM-70	960	32	90	285	83
Fraction 2-H	79	350	84	1300	31
Fraction 4-H	9.8	1500	45	5200	15

Table II. Amino acid and carbohydrate composition

			Urinary hCG in normal pregnancy	
	LM-hCG	*HM-hCG*	*Our laboratory**	*Other source†*
Amino acid (mol/100 mol residue)				
Lys	6.2	7.0	4.8	4.4
His	2.1	2.5	1.2	1.8
Arg	3.6	4.2	6.4	6.6
Asp	10.1	9.0	8.6	7.4
Thr	6.4	6.0	7.0	7.3
Ser	7.8	6.5	7.0	9.5
Glu	9.8	10.9	8.2	7.9
Pro	5.7	5.3	11.7	12.2
Gly	10.6	9.8	6.5	5.3
Ala	7.5	7.6	6.2	5.4
½ Cys	4.4	3.4	6.8	8.6
Val	6.3	7.2	9.2	7.9
Met	1.7	2.0	1.8	1.7
Ile	3.5	4.1	3.1	2.4
Leu	9.0	8.5	7.2	6.4
Thy	2.8	3.1	1.4	2.6
Phe	2.6	2.8	2.9	2.4
Carbohydrate (gm /100 gm protein)				
Total hexose	12.0	12.7	13.5	11.2
Total hexosamine	4.2	3.5	14.1	11.1
Sialic acid	0.3	0.5	9.5	9.0
Total carbohydrate	16.5	16.7	37.1	31.3

*The preparation in our laboratory (reference 8).
†Data taken from reference 9.

HM-hCG is similar to that of human chorionic luteinizing hormone and follicle-stimulating hormone, which were purified from the chorionic tissue of normal pregnancy by Tojo et al.[5] Canfield et al.[6] reported that the hCG recovered from urine of choriocarcinoma patients and normal pregnant individuals was not different in amino acid composition. The differences in amino acid contents of LM-hCG and HM-hCG from that of urinary hCG of normal pregnancy might be due to the fact that the former two were obtained directly from the tissue of origin.

Similarly, the carbohydrate compositions of LM-hCG and HM-hCG are almost the same. LM-hCG and HM-hCG have lower carbohydrate (16.5% and 16.7%, respectively) than urinary hCG of normal pregnancy (37.1%). The hexose content is nearly identical in all three, whereas the amino sugar contents of LM-hCG and HM-hCG appear to be approximately one fourth the amount in urinary hCG of normal pregnancy. Moreover, the amounts of sialic acid in LM-hCG and HM-hCG are remarkably less than that of urinary hCG of normal pregnancy. The amounts of carbohydrate are also considerably less than those of human chorionic luteinizing hormone and follicle-stimulating hormone previously described.[5]

LM-hCG and HM-hCG possess only about 10% of the biologic activity of urinary hCG of normal pregnancy. The biologic activity of highly purified urinary hCG of normal pregnancy is reported to be about 12,000 to 18,000 IU/mg.[7] It seems likely that the very low biologic activities of LM-hCG and HM-hCG are due to structural differences between these materials and the preparation of urinary hCG of normal pregnancy, since the latter had an appropriately high biologic activity (17,000 IU/mg) in the same in vivo test system. The low sialic acid contents of LM-hCG and HM-hCG might be responsible for their relatively low biologic activities. An alternative explanation for the low biologic activities of LM-hCG and HM-hCG is that they exist as precursors of hCG, which exist prior to the attachment of carbohydrate moieties, particularly sialic acid.

In conclusion, LM-hCG and HM-hCG have practically identical physicochemical, immunologic, and biologic properties. Both preparations have high levels of immunologic but low levels of biologic activity and essentially identical amino acid and carbohydrate compositions. It appears that moles of patients with high levels as well as those with low levels of urinary hCG immunoreactivity produce this form of hCG. Extracts of molar tissue from patients with high levels of urinary hCG immunoreactivity also contained other substances with apparent hCG immunoreactivity which were not found in tissue extracts from patients who excreted low levels of hCG immunoreactivity. This difference in molar tissue content of hCG immunoreactivity may account for the different levels excreted by these two types of patients. The nature of these other substances with hCG immunoreactivity will be the subject of future studies.

We wish to thank Drs. E. G. Armstrong and S. Birken (Departments of Pathology and Medicine, College of Physicians and Surgeons of Columbia University) for their review of this manuscript.

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CORRESPONDENCE

Use of atropine to differentiate normoxic from hypoxic bradycardia

To the Editors:

Parer (Parer JT. The effect of atropine on heart rate and oxygen consumption of the hypoxic fetus. AM J OBSTET GYNECOL 1984;148:1118) has again laid rest the notion that atropine (200 μg infused into fetal sheep) might alleviate hypoxia. However, within the article is the nucleus of a diagnostic test to differentiate hypoxic from normoxic bradycardia. An "atropine challenge test" should be able to sort out false positive from true late decelerations. Estimates are that as many as 50% of late fetal heart rate decelerations are false positive indicators of true fetal distress or uteroplacental insufficiency.[1] Scalp sampling is not always available or technically possible (long, closed cervix) to determine which half of these patients require urgent delivery. We have all undertaken cesarean section for late decelerations with no evidence of fetal hypoxia at delivery. Furthermore, in true uteroplacental insufficiency, the Po_2 should drop before acidemia occurs. If the fetus is hypoxic, by blocking the vagal component of late decelerations, atropine should result in a profound tachycardia, presumably as a result of unmasking the effect of catecholamines released in response to hypoxic stress, perhaps even before the pH drops. In a normoxic fetus there should be only a small rise in heart rate after vagal blockade with atropine. For further study I propose the management scheme shown here.

Persistent late decelerations
(early labor or positive contraction stress test)
(baseline fetal heart rate should be ≤160 bpm)
↓ ↓
Administer 200 μg of atropine intravenously
↓ ↓

Tachycardia	Minimal or no tachycardia (fetal heart rate ≤ 175 bpm)
Consistent with high catecholamines or myocardial depression, secondary to fetal hypoxia	Consistent with normoxia; perform pH to confirm if possible; observe
If conservative measures fail and pH determination is not possible, expedite delivery	

In a sense, this study has already been conducted in humans in Uruguay by Schifferli and Caldeyro-Barcia,[2] who noted that as the degree of asphyxia increases, the postatropine heart rate increases in a linear fashion. Atropine abolishes early and mild variable decelerations but not deep variable or true late decelerations. Apgar scores of 9 and 10 were found in infants with postatropine baseline heart rates of <175 bpm, and Apgar scores of 1 to 6 were found with postatropine heart rates >175 bpm. It is merely necessary (perhaps by monitoring creatine phosphokinase levels in Parer's model) to demonstrate that the postatropine tachycardia causes no myocardial damage, and an effective means of distinguishing the hypoxic fetus from the normoxic one is at hand.

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Reply to Brand

To the Editors:

Dr. Brand's suggestion is a valuable attempt to apply the findings of basic studies to clinical fetal surveillance. It is somewhat reminiscent of the suggestion of Goodlin and Haesslein[1] to abolish what they called "inappropriate fetal bradycardia" by the use of atropine. I am opposed to introducing Goodlin's suggestion into clinical obstetrics at this stage, and for many of the same reasons I would be opposed to Brand's test until more studies have been done, particularly in experimental animals.[2]

I would like to make some further comments on Brand's letter. All of us, he states, have undertaken cesarean sections for late decelerations, with no evidence of fetal hypoxia at delivery, and he quotes the approximately 50% false positive rate of using late decelerations as an index of depressing fetal hypoxia at delivery. Many clinicians now believe that one can reduce this inappropriate diagnosis markedly by relying heavily on the decrease or loss of fetal heart rate variability to determine which fetuses are becoming centrally asphyxiated.[3] The rationale for this is based on observations that there are two distinct types of late decelerations, one due to vagal reflex and the second due to transient hypoxic myocardial failure.[4] At the present time we believe these types can be distinguished by presence (for reflex late decelerations) or absence (for myocardial hypoxic late decelerations) of fetal heart rate variability. Clinical observations and some surveys[5] show that it is quite rare for fetuses to be born depressed when fetal heart rate variability is maintained, despite the presence of various periodic changes, including late decelerations. The physiologic basis for this observation has been extensively described.[6]

Brand states that in true uteroplacental insufficiency, the Po_2 should drop before acidemia occurs. This is not necessarily true of the respiratory component of acidosis. In studies where uterine blood flow is deliberately reduced we have seen an increase in Pco_2 as well as a decrease in Po_2.[7]

A further difficulty with Brand's proposed test concerns atropine dosage. In experimental sheep in order to reliably produce total fetal blockade one needs to administer 200 μg/kg intravenously to the fetus. Brand proposes 400 μg of atropine to the mother. Variations in rate and quantity absorbed may make fetal dosage quite unpredictable, thus casting doubt on the validity of a "negative" test.

The most serious objection to the test is that the duration of atropine's action can be such that its effect on heart rate may be prolonged. If so, this prevents the use of the fetal heart rate as a screen for asphyxia following the test. This would be an untenable situation given the fact that the patients in whom the test is to be used are already having "persistent late decelerations," and the cumulative effect of such intermittent stresses may result in serious asphyxia after the fetus has successfully "passed the test."

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The "lytic cocktail" induces recurrence of fits in the treatment of eclampsia

To the Editors:

We feel that the "lytic cocktail" must be banned in the treatment of eclampsia. In an effort to control 30 patients with eclamptic seizures, the Obstetrics Clinic of the Paulista Medical School administered a combination of chlorpromazine and promethazine (100 to 400 mg of each drug per 24 hours) and an intravenous priming dose of pethidine (100mg/24 hr), diluted in a 5% glucose solution. This mixture of drugs proved to be a "cocktail" that induced recurrence of seizures rather than stopping them (Table I).

We have divided the cases into four groups as a function of the grade of severity. Group A (Fig. 1) consisted of 12 patients with low arterial blood pressure levels who continued to have seizures throughout labor until their babies were delivered. Group B (Fig. 2) involved three patients who remained convulsive after delivery. Group C (Fig. 3) consisted of 11 eclamptic patients who experienced seizures during labor and the first 20

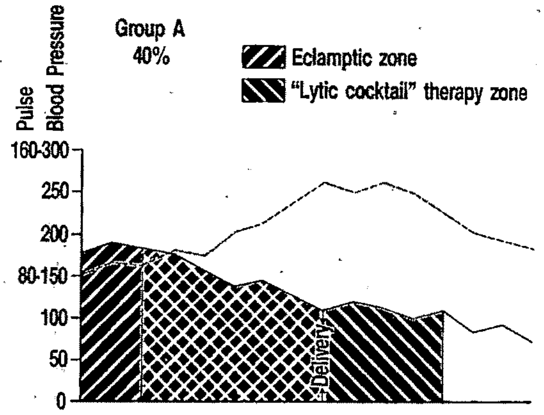

Fig. 1. Findings in group A.

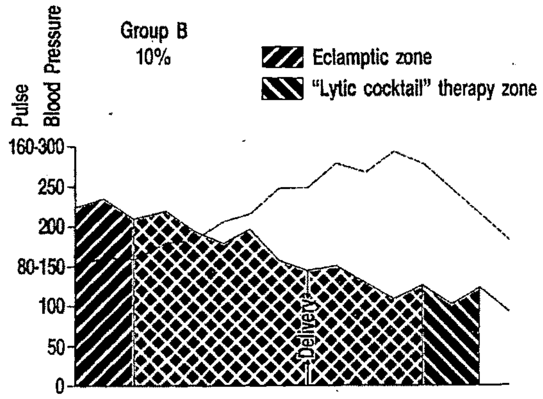

Fig. 2. Findings in group B.

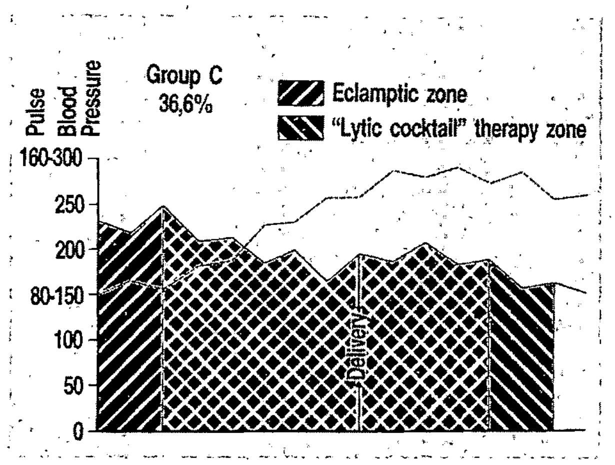

Fig. 3. Findings in group C.

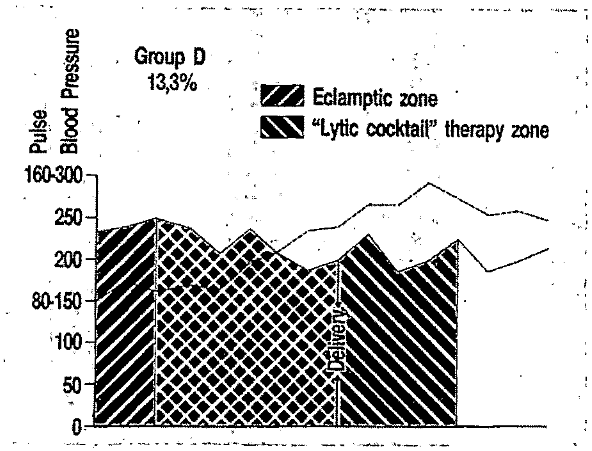

Fig. 4. Findings in group D.

Table I. Recurrence of seizures following administration of "lytic cocktail" in 30 patients

No.	0 to 2 seizures	More than 2 seizures	Fetal growth	Perinatal mortality			Maternal mortality	Delivery
				Stillbirth	Stillbirth during labor	Neonatal mortality		
Group A								
1	0	—	IUGR 2	—	—	—	0	Normal 2
2	0	—	IUGR	—	—	1	0	Forceps
3	0	—	Normal	—	—	—	0	Cesarean
4	1	—	Normal	—	—	—	0	Forceps
5	0	—	Preterm	—	—	1	0	Breech
6	0	—	—	1	—	—	0	Forceps
7	1	—	IUGR	—	—	1	0	Cesarean
8	—	4	IUGR	—	—	—	0	Cesarean
9	2	—	—	1	—	—	0	Normal
10	2	—	IUGR	—	—	—	0	Cesarean
11	—	4	IUGR	—	—	1	0	Cesarean
12	1	—	Normal	1	—	—	0	Normal
Group B								
13	2	—	IUGR	—	—	—	0	Normal
14	2	—	Normal	—	—	—	0	Cesarean
15	2	—	Preterm	—	1	—	0	Normal
Group C								
16	—	13	IUGR	—	—	—	0	Normal
17	1	—	Normal	—	—	—	0	Normal
18	—	9	—	1	—	—	0	Normal
19	1	—	IUGR	—	—	—	0	Normal
20	—	4	Normal	—	—	—	0	Breech
21	—	4	Normal	—	—	—	0	Normal
22	—	6	Normal	—	—	—	0	Normal
23	—	4	—	1	—	—	0	Normal
24	—	14	Normal	—	—	—	0	Cesarean
25	—	29	Normal	—	—	—	0	Cesarean
26	—	29	Normal	—	—	—	1	Cesarean
Group D								
27	2	—	IUGR	—	1	—	0	Forceps
28	—	29	—	1	—	—	0	Normal
29	—	39	—	1	—	—	0	Forceps
30	0	—	Normal	—	—	—	0	Normal

Occurrence of seizures: 0, 20%; 1, 16.7%; 2, 20%; 0-2, 56.6%; >2, 43.3%. Fetal growth, 58%; stillbirth, 22.5%; stillbirth during labor, 8.3%; neonatal mortality, 16.6%; maternal mortality, 3.3%; delivery, 45.1%. IUGR, Intrauterine growth retardation.

hours puerperium while being treated with the "lytic cocktail." Seizures stopped when this therapy was abandoned. The only case of maternal mortality occurred in the latter group. The patient suffered 29 seizures and died 2 hours after a cesarean section as she was receiving a blood transfusion for operative shock and 250 mg of thiopentane in an attempt to stop the seizures. There were four patients in group D (Fig. 4) whose seizures stopped after delivery. One patient in this group had 39 seizures. The rate of intravenous dripping was governed by patient response, and paradoxically, in some cases the increasing of dripping resulted in an increase in the number of convulsions.

It seems likely that the combination of phenothiazines and pethidine is a convulsant therapy in eclampsia. There was a reduction in systolic pressure (Figs. 1-4) without a relevant decrease in diastolic pressure in most of the eclamptic patients. These observations were made for a very elevated cardiac rate (pulse up to 160 bpm). These clinical data suggest that there is a decrease in blood flow in the brain. These findings reinforce the hypothesis of Sheehan and Lynch[1] that convulsions result from a vasomotor disturbance.

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REFERENCE

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Coincidence of congenital aplasia of the vagina with ear and facial anomalies

To the Editors:

I read with interest the article by Winer-Muram et al. (Winer-Muram HT, Muram D, Wilroy RS, Cupp C. The concurrence of facioauriculovertebral spectrum and the Rokitansky syndrome. AM J OBSTET GYNECOL 1984;149:569.) The statement that "the concurrence of the facioauriculovertebral spectrum and the Rokitansky syndrome has not been previously described" is not correct. A similar case has been published before.[1]

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REFERENCE

1. Willemsen WNP. Renal–skeletal–ear- and facial-anamolies in combination with the Mayer-Rokitansky-Küster (MRK) syndrome. Eur J Obstet Gynecol Reprod Biol 1982;14:121.

Reply to Willemsen

To the Editors:

This letter is being written in reply to Dr. Willemsen's comments about the article, The concurrence of facioauriculovertebral spectrum and the Rokitansky syndrome. The cases are similar, and indeed Dr. Willemsen's comments are entirely justified. Our patient was seen and written up in October, 1982, at which time we were unaware of Dr. Willemsen's article which had not been published as yet. The similarity of these two cases supports the hypothesis that a spectrum of mesenchymal defects indeed exists.

We would like to thank Dr. Willemsen for his comments.

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Prognostic significance of adenofibromatous pattern in ovarian epithelial malignancies

To the Editors:

I read with great interest the article of Malkasian et al. "Prognostic significance of histologic classification and grading of epithelial malignancies of the ovary" (AM J OBSTET GYNECOL 1984; 149:274), and would like to make the following comment.

Apart from stage, grade, and cell type, which have been studied and discussed by the authors, there is another factor that has not been mentioned at all, i.e., the presence or absence of adenofibromatous or cystadenofibromatous pattern. This histologic variant, which is more common with the serous cell type than with the other cell types of ovarian epithelial malignancies, has been listed as a separate subdivision in the World Health Organization classification.[1] Most probably, in cases with this histologic variant the malignant epithelium induces an increased stromal cellularity, and as a consequence of this, the tumor exhibits histologically a prominent fibrous stroma. It should be emphasized that the stroma does not show any degree of atypism or mitotic activity, thus ruling out the possibility of Müllerian adenosarcoma. Unfortunately, the prognostic significance of the presence or absence of adenofibromatous or cystadenofibromatous pattern in ovarian epithelial malignancies is still not known and yet has to be determined.

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REFERENCE

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Reply to Piura

To the Editors:

The presence of epithelial malignancies of the ovary in association with adenofibromas or buried within these lesions has been known for some time. The activity of these lesions appears to be associated with the grade of the epithelial malignancy and the stage of the disease present at the time of diagnosis and is unrelated to the presence or absence of the adenofibroma.

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Significance of fetal deceleration during antepartum heart rate testing

To the Editors:

Dashow and Read published in the January 15, 1984, issue an interesting study concerning clinical significance of fetal bradycardia during antepartum heart rate testing (Am J Obstet Gynecol 1984;148:187). We would like to make a few remarks concerning this study. The authors, for an unclear reason, keep calling bradycardia the pattern that they observed. We found it difficult to understand in how many of these 12 cases the pattern was of sporadic deceleration and in how many it was of baseline bradycardia. Needless to say, each would be of different significance.

Their conclusion regarding the significance as well as clinical management is based on 12 cases. This finding regarding antepartum fetal heart rate monitoring is not so rare. Druzin et al.[1] reported an incidence of 0.74%, and in our study it was 0.86%.[2] Therefore it is perplexing that the authors recommend a clinical management based on so few cases.

In our study[2] single sporadic deceleration was defined as a drop of fetal heart rate of at least 40 bpm below baseline lasting for at least 2 minutes. Out of 7202 nonstress tests, 62 exhibited this deceleration. In 48 of these cases, the single sporadic deceleration was followed by a normal reactive nonstress test. In 14 patients, nonstress test results following the single sporadic deceleration was nonreactive. In those cases, an oxytocin challenge test was performed and was negative in 10 cases and positive in four cases. In the latter four patients, labor was induced, and all required cesarean section because of severe fetal distress. The remaining 58 women were followed up at the High Risk Pregnancy Unit until spontaneous labor began. The interval from the diagnosis of the deceleration until delivery ranged from 1 to 28 days. In eight of the 58 cases fetal distress was observed during labor, and in four of the cases cesarean section was required. Fetal distress in labor was diagnosed in 12 cases (19.3%). This is ninefold more than in our general obstetric population. There were no cases of antepartum or intrapartum fetal death.

Since the obstetric sample of Dashow and Read is very selective, this may explain the high rate of antepartum fetal death (three of 12). In our series, in 32 of 62 cases the nonstress test was part of routine obstetric examination in otherwise normal pregnancy.

In conclusion, it is our opinion that the occurrence of single sporadic deceleration at gestational ages of 32 to 42 weeks is not an alarming sign providing the nonstress test and oxytocin challenge test are normal. However, the incidence of fetal distress during labor is significantly higher in fetuses with single sporadic deceleration as compared to the normal parturient population.

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REFERENCES

1. Druzin ML, Gratiacos J, Keegan KA, Paul RH. Antepartum fetal heart rate testing. VII. The significance of fetal bradycardia. Am J Obstet Gynecol 1981;139:194.
2. Hagay ZJ, Mazor M, Leiberman JR, Katz M, Insler V. The significance of single sporadic deceleration during a nonstress test. Eur J Obstet Gynecol Reprod Biol 1983;15:165.

Reply to Mazor et al.

To the Editors:

The interest in our article is greatly appreciated. We apologize for the confusion concerning the term "bradycardia"; however, we adopted this from Druzin et al.,[1] as we also felt it appropriate for this pattern change. The patterns seen in all 12 cases were sporadic changes as opposed to baseline bradycardia.

It is difficult to understand the perplexity felt concerning our conclusion and clinical management advice. We realize that with our study and that of Druzin et al.[1] there are few numbers cited. Given the significant outcomes, however, of these patients and the study cited by Hagay et al.,[2] we would not think it too radical to recommend that, when dealing with a combination of a high-risk situation and/or oligohydramnios and the aforementioned fetal heart rate change, a more intensive management program be utilized, i.e., more frequent antepartum testing (nonstress test, oxytocin challenge test, biophysical profile) or possibly delivery.

Since the end of our study three more of our patients have had the bradycardia pattern discussed. Two patients were at 41 weeks' gestation with inducible cervices and underwent nonstress tests because of late dates. Both had reactive nonstress tests and negative oxytocin challenge tests. Labor was induced 1 and 2 days after the bradycardia pattern, and both patients underwent cesarean section secondary to fetal distress manifested by late decelerations and significantly depressed scalp pH. Both had decreased but adequate amniotic fluid volumes on ultrasound examination before delivery and at rupture of membranes. Other than late dates, there was no obvious reason for the distress. The infants did well following delivery. The third patient had a nonstress test at 35 weeks' gestation because of decreased fetal movement. The nonstress test was reactive with one deceleration to 80 bpm for 1½ minutes from a baseline of 140 bpm. The oxytocin challenge test was negative. She was followed until 37 weeks' gestation with biweekly nonstress tests, which were reactive without bradycardia, and weekly biophysical profiles (three), which were all greater than 8. Fetal movements during the observation period were daily and cyclic. At 37 weeks she went into labor and underwent a cesarean section at 4 cm following severe variable decelerations. There were two tight wraps of nuchal cord. The infant did well following delivery.

In conclusion, it is our opinion that the previously mentioned sporadic bradycardia pattern is a significant finding and calls for more intensive monitoring and possibly expedient delivery.

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REFERENCES

1. Druzin ML, Gatacos J, Keegan KA, Paul RH. Antepartum fetal heart rate testing. VII. The significance of fetal bradycardia. AM J OBSTET GYNECOL 1981;139:194.
2. Hagay ZJ, Mazor M, Leiberman JR, Katz M, Insler V. The significance of single sporadic deceleration during a nonstress test. Eur J Obstet Gynecol Reprod Biol 1983;15:165.

Measurement of cervical dilatation

To the Editors:

This is with reference to the article of Fayemi et al., Internal and external cervical os dilatation with vaginal 15-methyl prostaglandin $F_{2\alpha}$ (AM J OBSTET GYNECOL 1983;146:219). I wish to point out a basic error in their data.

They measured cervical dilatation with the use of Hegar dilators. This means that the diameter of the cervical os was determined correct to the nearest millimeter. However, the mean cervical dilatation in Table I is presented in terms of a hundredth of a millimeter. This gives a spurious appearance of accuracy which is unwarranted and misleading.

By means of a calculator it is easy to compute averages to the third decimal place. However, since the raw figures themselves are accurate only to the nearest millimeter, the numbers after the decimal point are not significant, and the mean should have been rounded off to the nearest millimeter.

It is a basic principle of statistics that the mean cannot be more accurate than the raw data from which it is derived. This principle has been violated in order to present figures that look more accurate than they really are.

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Genetic counseling for low maternal serum α-fetoprotein

To the Editors:

An association between low maternal serum α-fetoprotein and fetal chromosome abnormalities was reported (Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum α-fetoprotein and fetal chromosomal abnormalities. AM J OBSTET GYNECOL 1984;148:886.) It has recently been suggested that both advanced maternal age and low maternal serum α-fetoprotein during a pregnancy provide independent measures of the risk for Down syndrome.[1]

Recently we provided genetic counseling to a 37-year-old woman, gravida 2, para 1. She was initially offered amniocentesis counseling on the basis of advanced maternal age but cancelled the appointment because she had decided against having the procedure. She did agree to a maternal serum α-fetoprotein screening, however, which was less than 0.4 multiples of the median for her gestational age.

On the basis of this result, she and her husband were counseled that their true risk for a trisomic fetus was probably higher than the 2% risk based on age alone, although we were not able to furnish a more accurate risk figure. The couple then reconsidered their original decision and decided to undergo amniocentesis. Fetal karyotype indicated trisomy 21, and they elected to terminate the pregnancy.

Although the mechanism for the low α-fetoprotein levels in pregnancies with Down syndrome and other chromosome abnormalities is not yet known, other laboratories have noted this phenomenon.[2] It is hoped that risk tables will be constructed with combined data on both maternal age and maternal serum α-fetoprotein to allow more accurate genetic counseling and to identify a higher percentage of trisomic pregnancies.

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REFERENCES

1. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984;1:926-929.
2. Guibaud S, Bonnet-Capela M, Germain D, Dumont M, Thoulon JM, Berland M. Prenatal screening for Down syndrome. Lancet 1984;1:1359-1360.

Roster of American obstetric and gynecologic societies*

(Appears annually in the January 1 issue)

American Board of Obstetrics and Gynecology, Inc. (1930) *President,* Leo J. Dunn. *Executive Director,* James A. Merrill, 4507 University Way N.E. #204, Seattle, Wash. 98105.

American College of Obstetricians and Gynecologists. (1951) *President,* Luella Klein. *Secretary,* Ezra C. Davidson, Jr., 600 Maryland Ave., S.W., Ste. 300E, Washington, D. C. 20024-2588. Meeting, May 13-16, 1985.

American Gynecological and Obstetrical Society. *President,* Joseph Seitchik. *Secretary,* Richard F. Mattingly, Medical College of Wisconsin, 8700 W. Wisconsin Ave., Milwaukee, WI 53226. Meeting, Sept. 4-7, 1985.

Central Association of Obstetricians and Gynecologists. (1929) *President,* George D. Malkasian, Jr. *Secretary,* James H. Maxwell, 320 E. Fontanero, Ste. 106, Colorado Springs, Colo. 80907. Meeting, Oct. 10-12, 1985.

Pacific Coast Obstetrical and Gynecological Society. (1931) *President,* Edward C. Hill. *Secretary,* Walter S. Keifer, 1145 Broadway, Seattle, Wash. 98122. Meeting, Sept. 29–Oct. 4, 1985.

Society for Gynecologic Investigation. *President,* William N. Spellacy. *Secretary,* John M. Bissonnette, Rm. 426, Research Bldg., Oregon Health Sciences University, Portland, Ore. 97201. Meeting, March 20-23, 1985.

Society of Obstetricians and Gynaecologists of Canada. (1945) *President,* Betty J. Poland. *Secretary,* Patrick T. Mohide, McMaster University, Faculty of Health Sciences, Dept. of Ob/Gyn, 1200 Main St. West, Rm 4D7, Hamilton, Ont., Canada L8N 3Z5.

South Atlantic Association of Obstetricians and Gynecologists. (1938) *President,* William T. Mixson. *Secretary,* Hobart C. Hortman, Jr., 10 Hospital Circle, Rome, Ga. 30161. Meeting, Jan. 27-30, 1985.

American Association of Pro Life Obstetricians and Gynecologists.

Atlanta Obstetrical and Gynecological Society. (1953)

Bronx Gynecological and Obstetrical Society. *President,* John T. Parente. *Secretary,* George Kleiner, 3424 Kossuth Ave., Bronx, N. Y. 10467. Meetings, third Monday of each month.

Brooklyn Gynecological Society, Inc. *President,* Thomas Manzo. *Secretary,* Gwen Gentile, Box 24, Downstate Medical Center, Brooklyn, N. Y. 11203. Meetings, Jan. 16, Feb. 20, March 20, April 17, and May 15, 1985.

Buffalo Gynecologic & Obstetric Society. (1945) *President,* Norman G. Courey. *Secretary,* Camille M. Perla, 77 Livingston St., Buffalo, N. Y. 14213. Meetings, Jan. 8, Feb. 12, March 12, and April 2, 1985.

Chicago Gynecological Society. *President,* George D. Wilbanks. *Secretary,* Melvin V. Gerbie, 707 N. Fairbanks Ct., Chicago, Ill. 60611. Meetings, third Friday of Oct.-June.

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*Changes, omissions, and corrections must be received by the publisher two months in advance (by November 1) for the Roster. Please address the Journal Editing Department, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. The number after the society's name is the year of founding. For further information, address the respective secretaries.

Cleveland Society of Obstetricians and Gynecologists. *President,* Matthew Biscotti. *Secretary,* Jeffrey C. Seiler, 9500 Euclid Ave., Cleveland, Ohio 44106. Meetings, Jan. 16, Feb. 20, March 20, and May 29, 1985.

Columbus Obstetric and Gynecologic Society.

East Bay Gynecological Society.

Florida Obstetric and Gynecologic Society.

Georgia Obstetrical and Gynecological Society, Inc. (1950)

Infectious Disease Society for Obstetrics and Gynecology. (1976) *President,* Marvin S. Amstey. *Secretary,* Richard L. Sweet, Rm. 6J2, San Francisco General Hospital, San Francisco, Calif. 94110. Meeting, Aug 7-10, 1985.

Kansas City Gynecological Society. (1932) *President,* William J. Huse. *Secretary,* Carl R. Slaughter, 5601 N.E. Antioch Rd., Gladstone, MO 64119. Meetings, third Thursday of Jan., March, May, Sept., and Nov.

Little Rock Gynecological Society, Inc.

Memphis and Shelby County Obstetrical and Gynecologic Society. *President,* Jesse Woodall. *Secretary,* Frank W. Ling, 800 Madison, Dept. Ob/Gyn, University of Tennessee, Memphis, Tenn. 38163. Meetings, on called basis.

Miami Obstetrical and Gynecological Society. (1946) *President,* Richard Corson. *Secretary,* Chester Morris, 660 N.E. 95th St., Miami Shores, Fla. 33138. Meetings, second Thursday of Jan., March, and May.

Milwaukee Gynecological Society. (1951) *President,* Michael Jaekels. *Secretary,* Robert H. Herzog, 850 Elm Grove Rd., Elm Grove, Wisc. 53122. Meetings, Feb. 11, March 11, and April 15, 1985.

Montana Section American College of Obstetricians and Gynecologists. (1948) *President,* John T. Browne. *Secretary,* Thomas C. Olson, P.O. Box 35100, Billings, MT 59107. Meeting, Feb. 28-March 2.

New Jersey Obstetrical and Gynecological Society. (1947) *President,* Robert Malatesta. *Secretary,* R. Richard Messick, 2 Princess Rd., Lawrenceville, N. J. 08648. Meeting, June 7, 1985.

New York Gynecological Society, Inc. *President,* Kevin Reilly. *Secretary,* David F. James, 45 East 85th St., New York, N. Y. 10028. Meetings, Jan. 23, March 27, May 22, and Oct. 23, 1985.

North Carolina Obstetrical and Gynecological Society.

North Dakota Society of Obstetrics and Gynecology. (1938) *President,* Tom Jacobson Hettinges. *Secretary,* S. P. Christensen, 737 Broadway, Fargo, N. D. 58123. Meeting, Sept. 6-7, 1985.

Obstetrical Society of Philadelphia. (1868) *President,* Luigi Mastroianni. *Secretary,* Jan Schneider, Medical College of Pennsylvania, 3300 Henry Ave., Philadelphia, Pa. 19129. Meetings, second Thursday Oct.-May.

Oklahoma City Obstetrical and Gynecological Society. (1940) *President,* Mayo Gilson. *Secretary,* Michael Seikel, 3131 N.W. Expressway, Oklahoma City, Okla. 73112. Meetings, second Monday of Feb., March, and May.

Peninsula Gynecologic Society.

St. Louis Gynecological Society. (1877) *President,* Gary Sheldon. *Secretary,* Jerry Middleton, 23 Clermont Ln., St. Louis, Mo. 63124. Meetings, third Thursday of Feb. and April.

San Antonio Obstetrical and Gynecological Society.

San Diego Gynecological Society. (1929) *President,* Kenneth D. Crippen. *Secretary,* Elizabeth Wilder, 4647 Zion Ave., San Diego, Calif. 92120. Meetings, fourth Tuesday of every month, Sept.-May.

San Francisco Gynecological Society. (1929) *President,* George Winch. *Secretary,* Gordon Peacock, 3878 California St., San Francisco, Calif. 94114. Meetings, by invitation.

Schufelt Gynecologic Society of Santa Clara Valley. (1947)

Seattle Gynecologic Society. *President,* Donald C. Smith. *Executive Secretary,* Dept. Ob-Gyn (RH-20), University Hospital, Seattle, Wash. 98195. Meeting, Sept. 20-21, 1985.

Texas Association of Obstetricians and Gynecologists. (1931) *President,* George B. Coale III. *Secretary,* Robert H. Wernecke, 1301 West 38th St., #403, Austin, Tex. 78705. Meeting, March 6-8, 1985.

Virginia Obstetrical and Gynecological Society. *President,* Clifford H. Fox. *Secretary,* Walter M. Zirkle, Medical Arts Bldg., Harrisonburg, Va. 22801. Meeting, Spring, 1985.

Washington Gynecological Society.

Wisconsin Obstetrical and Gynecological Society. *President,* John W. Utrie. *Secretary,* William Martens, 10425 W. North Ave., Milwaukee, Wisc. 53226. Meeting, July 19-20, 1985.

Youngstown Society of Obstetricians and Gynecologists.

Books received

Baby Hunger. Every Woman's Longing for a Baby. Lois Leiderman Davitz. 136 pages. Minneapolis, 1984, Winston Press. $7.95 (soft cover).

Disease and Fertility. Joseph A. McFalls, Jr., and Marguerite Harvey McFalls. 593 pages, illustrated. Orlando, Florida, 1984, Academic Press Inc. $69.50/£49.00.

Drug Prescribing in Pregnancy. Beatrice Krauer, Felix Krauer, and Frank Hytten. 188 pages, illustrated. New York, 1984, Churchill Livingstone, Inc. $19.00 (soft cover).

Endocrinology, 1984. Theodore B. Schwartz and Will G. Ryan. 427 pages, illustrated. Chicago, 1984, Year Book Medical Publishers, Inc. No price listed.

Ontogeny of Bonding—Attachment. Progress in Reproductive Biology and Medicine, Volume II. P. O. Hubinont. 178 pages, illustrated. Basel, Switzerland, 1984, Karger. $87.00.

Progress in Obstetrics and Gynecology. Volume 4. John Studd. 395 pages, illustrated. New York, 1984, Churchill Livingstone, Inc. $26.00 (soft cover).

Sexually Transmitted Diseases. King K. Holmes, Per-Anders Mårdh, P. Frederick Sparling, and Paul J. Wiesner. 1079 pages, illustrated. New York, 1984, McGraw-Hill Book Company. $50.00.

Suicide in Pregnancy. Edited by George J. Kleiner and Wilma Markus Greston. 213 pages, illustrated. Boston, 1984, Wright PSG, Inc. No price listed.

Ultrasound in Perinatal Care. M. J. Bennett, Guest Editor. 187 pages, illustrated. New York, 1984, John Wiley & Sons, Inc. $37.00.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Gynecologic Surgery—Current and Future Issues, The George Washington University School of Medicine, March 14-16, 1985. Sponsored by the Department of Obstetrics and Gynecology. For further information contact: Arnold Friedman, M.D., Department of Obstetrics and Gynecology, 2150 Pennsylvania Ave., N.W., Washington, D. C. 20037. Tel.: (202) 676-4374.

Management of Complications of Gynecologic Surgery and Urology, April 26-27, 1985, The Drake Hotel, Chicago, Illinois. Sponsored by The University of Chicago Pritzker School of Medicine. For further information contact: CME Office, The University of Chicago, Box 139, 5841 Maryland, Chicago, IL 60637. Tel.: (312) 962-1056.

Post Graduate Course in Essential Colposcopy and Gynecologic Laser Surgery, February 15-16, 1985, Westin Oaks Hotel, The Galleria, Houston, Texas. For further information contact: Dr. V. C. Wright, 887 Richmond St., London, Ontario, Canada N6A 3J1. Tel.: (519) 438-1411.

Eighth Annual Mid-Winter Symposium in OB/GYN, February 27–March 1, 1985, Scottsdale Hilton Hotel, Scottsdale, Arizona. Co-sponsored by the Phoenix OB/GYN Society and the Department of OB/GYN, Maricopa Medical Center. For further information contact: The Secretary, Phoenix OB/GYN Society, 3412 N. Fourth Ave., Phoenix, AZ 85013. Tel.: (602) 241-1917.

IPA/HMO Investment and Participation, Northeast Ohio Physician Meeting for Doctors of Obstetrics and Gynecology, Wednesday, February 20, 1985, Western Reserve Health Plan, 19101 Villaview Road, Suite 207, Cleveland, OH 44119. For further information contact: Provider Relations Department at (216) 486-0152.

OB/GYN Courses: "Endocrinology and High Risk Pregnancy," Rome/Dubrovnik, April 17-28, 1985; "Genetics and Ultrasound," Dubrovnik/Rome, April 17-28, 1985. For further information contact: Dr. Robert C. Cefalo, University of North Carolina, 214 McNider Building, Chapel Hill, NC 27514. Tel.: (919) 967-5685.

An Update in Obstetrics and Gynecology for the Practicing Specialist, February 11-15, 1985, Beverly Hilton Hotel, Beverly Hills, California. For further information contact: Obstetrical and Gynecological Assembly, 5820 Wilshire Blvd., Suite 500, Los Angeles, CA 90036. Tel.: (213) 937-5514.

Update in Diagnosing & Treating OB/GYN Infections, March 9-10, 1985, Holiday Inn, Center Strip, Las Vegas, Nevada. For further information contact: GYNE-CME Fund, 2570 S. Bruce St., Suite # B-4, Las Vegas, NV 89109. Tel.: (702) 732-2590.

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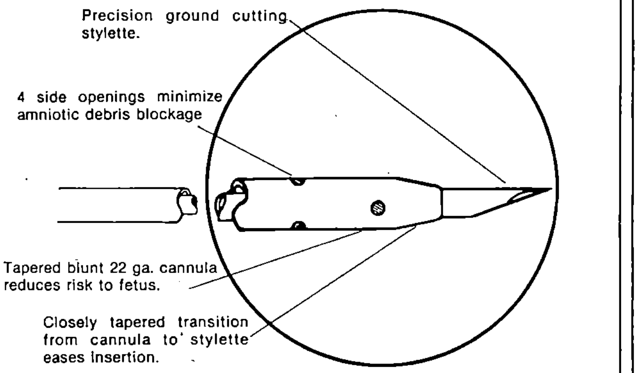
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CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions. 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000, ages 35-44—33/100,000, ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7 fold increase in risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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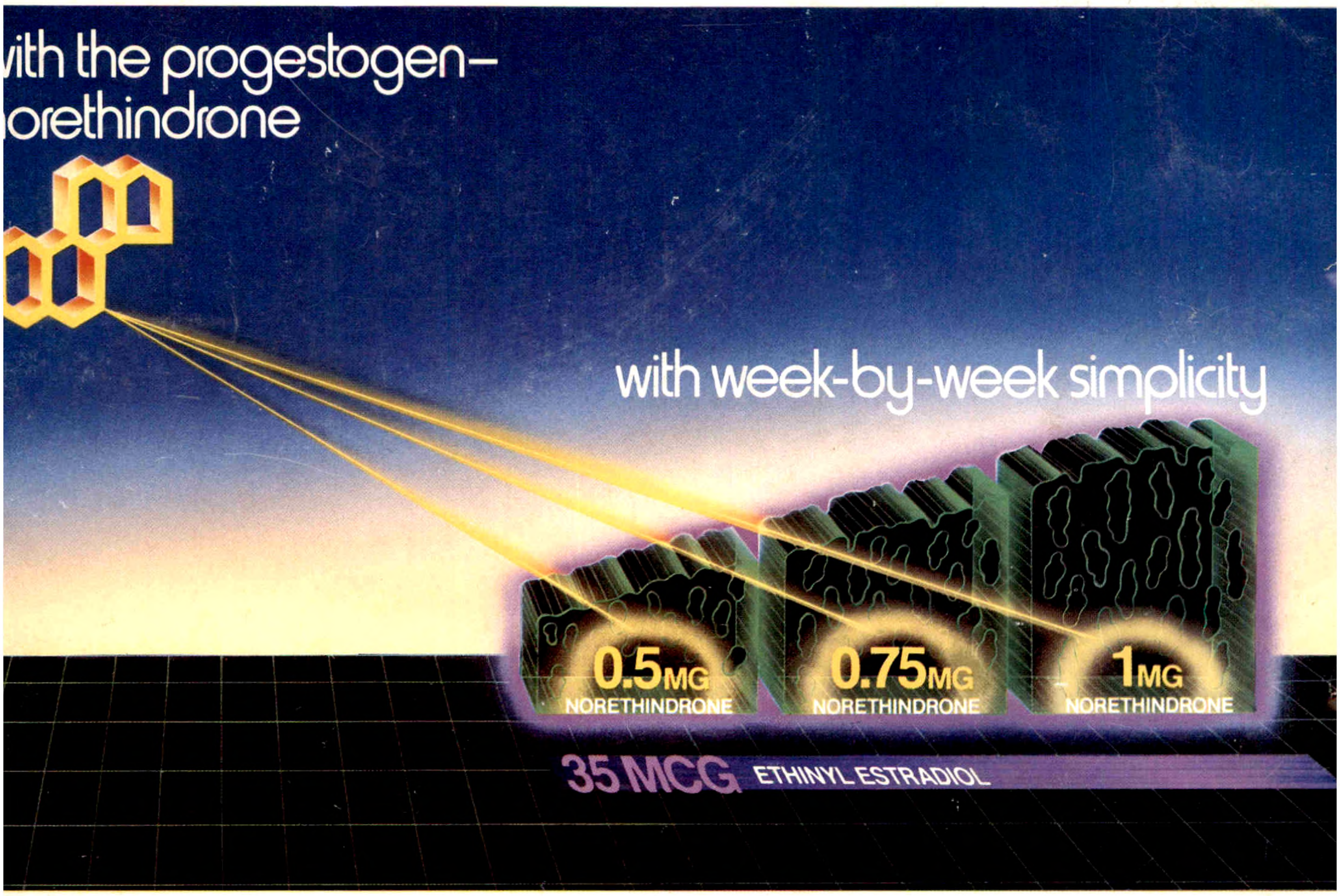

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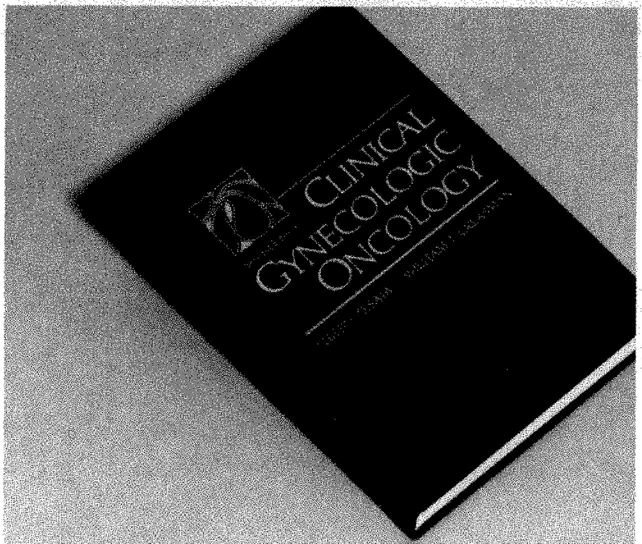

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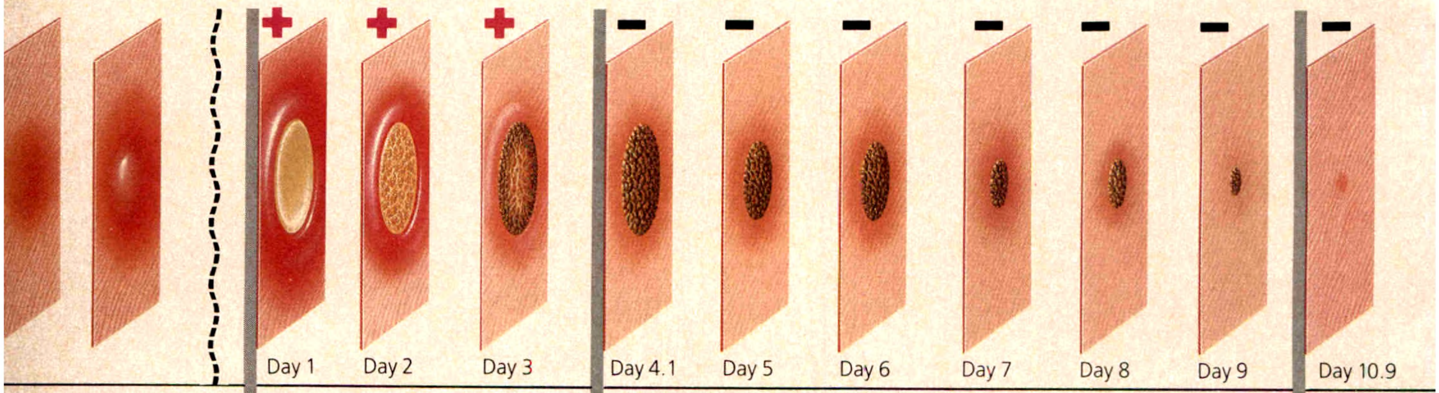

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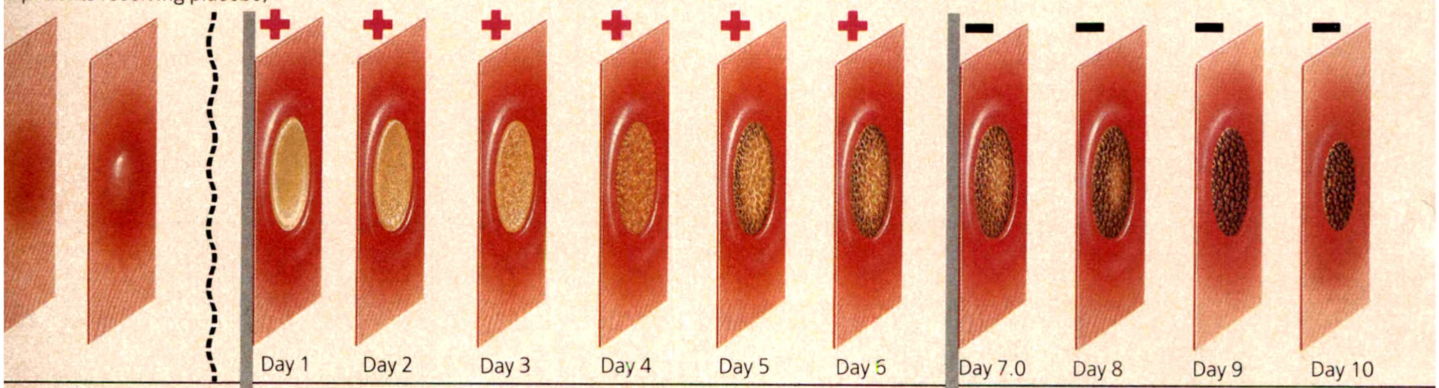

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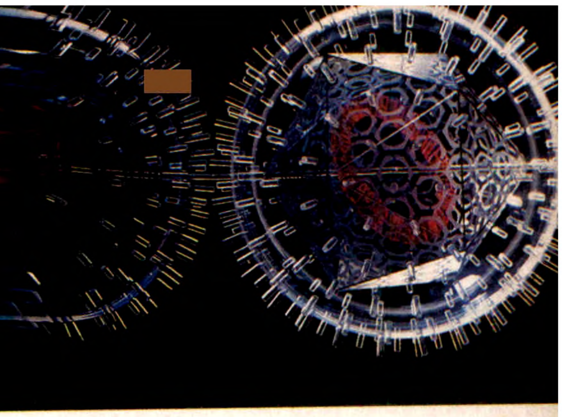
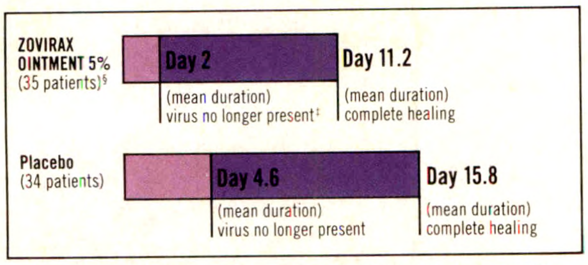


An additional double-blind randomized study confirms the efficacy of ZOVIRAX Ointment 5% in patients with initial infections who presented within 6 days of onset of lesions[6*]



What's more, either form of the medication — I.V. or topical — rapidly achieves potent antiviral effects. Within hours, the I.V. form reaches plasma concentration levels that are substantially higher than the 50% inhibitory dose required for Herpes simplex virus types 1 and 2.[4]

And safety is not jeopardized by the antiviral potency of ZOVIRAX, which has a highly selective — virus-activated — mode of action.

By prescribing early intervention with ZOVIRAX you can dramatically —

- ☐ alter the time the virus is present at the lesion site
- ☐ alter the number of new lesions formed
- ☐ alter the time to crusting and complete healing
- ☐ alter the time to cessation of pain

*For the best clinical response, ZOVIRAX Ointment 5% therapy should be initiated as early as possible following onset of signs and symptoms.

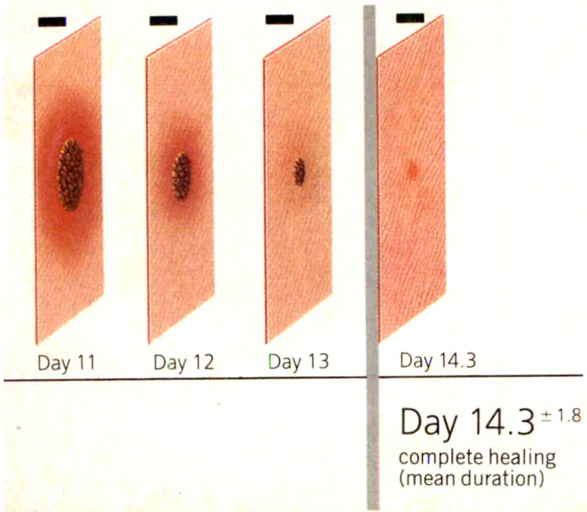
†Patients in this study applied ZOVIRAX 4 times daily for 7 days; recommended therapy is 6 times daily for 7 days.

‡Virus no longer present is a measure of the antiviral effect of ZOVIRAX and means that the replication and shedding of the virus have ceased at the sites cultured.

§Antiviral and complete healing results in this study are based on ZOVIRAX applied 4 to 6 times a day for 7 days; the recommended therapy for ZOVIRAX Ointment 5% is 6 times a day for 7 days.

References: 1. Corey L: Treatment of severe first episodes of genital herpes infections with intravenous acyclovir. Scientific exhibit at the Annual Meeting of the American College of Obstetricians and Gynecologists, Dallas, TX, Apr 26-29, 1982. 2. Mindel A, Adler MW, Sutherland S, et al: Intravenous acyclovir treatment for primary genital herpes. *Lancet* 1982; 1: 697-700. 3. Fife KH, Corey L, Keeney RE, et al: Double blind placebo controlled trial of intravenous acyclovir for severe primary genital herpes. Abstract No 29 from 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Nov 4-6, 1981. 4. Data on file, Burroughs Wellcome Co. 5. Corey L, Nahmias AJ, Guinan ME, et al: A trial of topical acyclovir in genital herpes simplex virus infections. *N Engl J Med* 1982; 306 (June 3): 1313-1319. 6. Corey L, Benedetti JK, Critchlow CW, et al: Double-blind controlled trial of topical acyclovir in genital herpes simplex virus infections. *Am J Med* (special issue), July 20, 1982, pp 326-334.

Please see brief summary on last page of this advertisement.



Day 14.3 $^{\pm 1.8}$
complete healing
(mean duration)

For the severe episode

ZOVIRAX® (ACYCLOVIR SODIUM) Sterile Powder FOR INTRAVENOUS INFUSION ONLY

INDICATIONS AND USAGE: Zovirax Sterile Powder is indicated for the treatment of initial and recurrent mucosal and cutaneous Herpes simplex (HSV-1 and HSV-2) infections in immunocompromised adults and children. It is also indicated for severe initial clinical episodes of herpes genitalis in patients who are not immunocompromised.

These indications are based on the results of several double-blind, placebo-controlled studies which evaluated the drug's effect on virus excretion, complete healing of lesions, and relief of pain.

Herpes Simplex Infections in Immunocompromised Patients

A multicenter trial of Zovirax Sterile Powder at a dose of 250 mg/M² every 8 hours (750 mg/M²/day) for 7 days was conducted in 97 immunocompromised patients with oro-facial, esophageal, genital and other localized infections (50 treated with Zovirax and 47 with placebo). Zovirax significantly decreased virus excretion, reduced pain, and promoted scabbing and rapid healing of lesions.[1,2,3]

Initial Episodes of Herpes Genitalis

A controlled trial was conducted in 28 patients with severe initial episodes of herpes genitalis with a Zovirax dosage of 5 mg/kg every 8 hours for 5 days (12 patients treated with Zovirax and 16 with placebo). Significant treatment effects were seen in elimination of virus from lesions and in reduction of healing times.[4]

In a similar study, 15 patients with initial episodes of genital herpes were treated with Zovirax 5 mg/kg every 8 hours for 5 days and 15 with placebo. Zovirax decreased the duration of viral excretion, new lesion formation, duration of vesicles and promoted more rapid healing of all lesions.[5]

Diagnosis

The use of appropriate laboratory diagnostic procedures will help to establish the etiologic diagnosis. Positive cultures for Herpes simplex virus offer a reliable means for confirmation of the diagnosis. In initial episodes of genital herpes, appropriate examinations should be performed to rule out other sexually transmitted diseases. Whereas cutaneous lesions associated with Herpes simplex infections are often characteristic, the finding of multinucleated giant cells in smears prepared from lesion exudate or scrapings may assist in the diagnosis[6]

CONTRAINDICATIONS: Zovirax Sterile Powder is contraindicated for patients who develop hypersensitivity to the drug.

WARNINGS: Zovirax Sterile Powder is intended for intravenous infusion only, and should not be administered topically, intramuscularly, orally, subcutaneously, or in the eye. Intravenous infusions must be given over a period of at least 1 (one) hour to prevent renal tubular damage (see PRECAUTIONS and DOSAGE AND ADMINISTRATION).

PRECAUTIONS:

General: The recommended dosage, frequency and length of treatment should not be exceeded (See DOSAGE AND ADMINISTRATION).

Although the aqueous solubility of acyclovir sodium (for infusion) is >100 mg/ml, precipitation of acyclovir crystals in renal tubules can occur if the maximum solubility of free acyclovir (2.5 mg/ml at 37°C in water) is exceeded or if the drug is administered by bolus injection. This complication causes a rise in serum creatinine and blood urea nitrogen (BUN), and a decrease in renal creatinine clearance. Ensuing renal tubular damage can produce acute renal failure.

Abnormal renal function (decreased creatinine clearance) can occur as a result of acyclovir administration and depends on the state of the patient's hydration, other treatments, and the rate of drug administration. Bolus administration of the drug leads to a 10% incidence of renal dysfunction, while in controlled studies, infusion of 5 mg/kg (250 mg/M²) over an hour was associated with a lower frequency — 4.6%. Concomitant use of other nephrotoxic drugs, pre-existing renal disease, and dehydration make further renal impairment with acyclovir more likely. In most instances, alterations of renal function were transient and resolved spontaneously or with improvement of water and electrolyte balance, drug dosage adjustment or discontinuation of drug administration. However, in some instances, these changes may progress to acute renal failure.

Administration of Zovirax by intravenous infusion must be accompanied by adequate hydration. Since maximum urine concentration occurs within the first 2 hours following infusion, particular attention should be given to establishing sufficient urine flow during that period in order to prevent precipitation in renal tubules.

When dosage adjustments are required they should be based on estimated creatinine clearance (See DOSAGE AND ADMINISTRATION).

Approximately 1% of patients receiving intravenous acyclovir have manifested encephalopathic changes characterized by either lethargy, obtundation, tremors, confusion, hallucinations, agitation, seizures or coma. Zovirax should be used with caution in those patients who have underlying neurologic abnormalities and those with serious renal, hepatic, or electrolyte abnormalities or significant hypoxia. It should also be used with caution in patients who have manifested prior neurologic reactions to cytotoxic drugs or those receiving concomitant intrathecal methotrexate or interferon.

Exposure of HSV isolates to acyclovir *in vitro* can lead to the emergence of less sensitive viruses. These viruses usually are deficient in thymidine kinase (required for acyclovir activation) and are less pathogenic in animals. Similar isolates have been observed in 6 severely immunocompromised patients during the course of controlled and uncontrolled studies of intravenously administered Zovirax. These occurred in patients with congenital severe combined immunodeficiencies or following bone marrow transplantation. The presence of these viruses was not associated with a worsening of clinical illness and, in some instances, the virus disappeared spontaneously. The possibility of the appearance of less sensitive viruses must be borne in mind when treating such patients. The relationship between the *in vitro* sensitivity of herpesviruses to acyclovir and clinical response to therapy has yet to be established.

Drug Interactions: Co-administration of probenecid with acyclovir has been shown to increase the mean half-life and the area under the concentration-time curve. Urinary excretion and renal clearance were correspondingly reduced. Clinical experience has identified no other significant interactions resulting from administration of other drugs concomitantly with Zovirax Sterile Powder.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of 50, 150 and 450 mg/kg given by gavage. There was no statistically significant difference in the incidence of tumors between treated and control animals, nor did acyclovir appear to shorten the latency of tumors. In 2 *in vitro* cell transformation assays, used to provide preliminary assessment of potential oncogenicity in advance of these more definitive lifetime bioassays in rodents, conflicting results were obtained. Acyclovir was positive at the highest dose used in one system and the resulting morphologically transformed cells formed tumors when inoculated into immunosuppressed, syngeneic, weanling mice. Acyclovir was negative in another transformation system.

No chromosome damage was observed at maximum tolerated parenteral doses of 100 mg/kg acyclovir in rats or Chinese hamsters; higher doses of 500 and 1000 mg/kg were clastogenic in Chinese hamsters. In addition, no activity was found in a dominant lethal study in mice. In 9 of 11 microbial and mammalian cell assays, no evidence of mutagenicity was observed. In 2 mammalian cell assays (human lymphocytes and L5178Y mouse lymphoma cells *in vitro*), positive responses for mutagenicity and chromosomal damage occurred, but only at concentrations at least 25 times the acyclovir plasma levels achieved in man.

Acyclovir does not impair fertility or reproduction in mice at oral doses up to 450 mg/kg/day. In female rabbits treated subcutaneously with acyclovir subsequent to mating, there was a statistically significant decrease in implantation efficiency but no concomitant decrease in litter size at a dose of 50 mg/kg/day.

Pregnancy: Teratogenic Effects. Pregnancy Category C. Acyclovir was not teratogenic in the mouse (450 mg/kg/day, p.o.), rabbit (50 mg/kg/day, s.c.) or rat (50 mg/kg/day, s.c.).

Although maximum tolerated doses were tested in the teratology studies, the plasma levels obtained did not exaggerate maximum plasma levels that might occur with clinical use of intravenous acyclovir.

There have been no adequate and well-controlled studies in pregnant women. Acyclovir should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Zovirax is administered to a nursing woman.

ADVERSE REACTIONS: The most frequent adverse reactions reported during controlled clinical trials of Zovirax in 64 patients were inflammation or phlebitis at the injection site following infiltration of the I.V. fluid in 9 (14.0%), transient elevations of serum creatinine in 3 (4.7%), and rash or hives in 3 (4.7%). Less frequent adverse reactions were diaphoresis, hematuria, hypotension, headache and nausea, each of which occurred in 1 patient (1.6%). Of the 63 patients receiving placebo, 3 (4.8%) experienced inflammation/phlebitis and 3 (4.8%) experienced rash or itching. Hematuria and nausea were experienced by placebo recipients at the same frequency.

Among 51 immunocompromised patients, one, a bone marrow transplant recipient with pneumonitis, developed seizures, cerebral edema, coma and expired with changes consistent with cerebral anoxia on postmortem biopsy; another immunocompromised patient exhibited coarse tremor and clonus.

Additional adverse reactions were reported in uncontrolled trials. The most frequent adverse reaction was elevated serum creatinine. This occurred in 9.8 percent of patients, usually following rapid (less than 10 minutes) intravenous infusion. Less frequent adverse experiences were thrombocytosis and jitters, each in 0.4% of patients.

Approximately 1% of patients receiving intravenous acyclovir have manifested encephalopathic changes characterized by either lethargy, obtundation, tremors, confusion, hallucinations, agitation, seizures or coma (see PRECAUTIONS).

OVERDOSAGE: No acute massive overdosage of the intravenous form has been reported.

Doses administered to humans have been as high as 1200 mg/M² (28 mg/kg) three times daily for up to two weeks. Peak plasma concentrations have reached 80 μg/ml. Precipitation of free acyclovir in renal tubules may occur when the solubility in the intratubular fluid is exceeded (see PRECAUTIONS). Acyclovir is dialyzable. In the event of acute renal failure and anuria, the patient may benefit from hemodialysis until renal function is restored (see DOSAGE AND ADMINISTRATION).

DOSAGE AND ADMINISTRATION: CAUTION — RAPID OR BOLUS INTRAVENOUS AND INTRAMUSCULAR OR SUBCUTANEOUS INJECTION MUST BE AVOIDED.

Dosage: ***MUCOSAL AND CUTANEOUS HERPES SIMPLEX (HSV-1 and HSV-2) INFECTIONS IN IMUNOCOMPROMISED PATIENTS*** — 5 mg/kg infused at a constant rate over 1 hour every 8 hours (15 mg/kg/day) for 7 days in adult patients with normal renal function. In children under 12 years of age, more accurate dosing can be attained by infusing 250 mg/M² at a constant rate over 1 hour, every 8 hours (750 mg/M²/day) for 7 days.

SEVERE INITIAL CLINICAL EPISODES OF HERPES GENITALIS — The same dose given above — administered for 5 days.

Therapy should be initiated as early as possible following onset of signs and symptoms.

PATIENTS WITH ACUTE OR CHRONIC RENAL IMPAIRMENT: Refer to DOSAGE AND ADMINISTRATION section for recommended doses, and adjust the dosing interval as indicated in the table below.

Creatinine Clearance (ml/min/1.73M²)	Dose (mg/kg)	Dosing Interval (hours)
>50	5	8
25-50	5	12
10-25	5	24
0-10	2.5	24

Hemodialysis: For patients who require dialysis, the mean plasma half-life of acyclovir during hemodialysis is approximately 5 hours. This results in a 60% decrease in plasma concentrations following a 6 hour dialysis period. Therefore, the patient's dosing schedule should be adjusted so that a dose is administered after each dialysis.

References: 1. C.D. Mitchell, *et al.*, Lancet *1*(8235): 1389-1392, Jun. 27, 1981. 2. J.C. Wade, *et al.*, Ann. Intern. Med. *96*(3): 265-269, Mar. 1982. 3. J.D. Meyers, *et al.*, Am. J. Med. *73*(1A): 229-235, Jul. 20, 1982. 4. Data on file, Burroughs Wellcome Co. 5. A. Mindel, *et al.*, Lancet *1*(8274): 697-700, Mar. 27, 1982. 6. Z.M. Naib, *et al.*, Cancer Res. *33*:1452-1463, 1973.

For the less-severe episode

ZOVIRAX® (ACYCLOVIR) Ointment 5%

INDICATIONS AND USAGE: Zovirax (Acyclovir) Ointment 5% is indicated in the management of initial herpes genitalis and in limited nonlife-threatening cutaneous Herpes simplex virus infections in immunocompromised patients. In clinical trials of initial herpes genitalis, Zovirax Ointment 5% has shown a decrease in healing time and in some cases a decrease in duration of viral shedding and duration of pain. In studies in immunocompromised patients with mainly herpes labialis, there was a decrease in duration of viral shedding and a slight decrease in duration of pain.

By contrast, in studies of recurrent herpes genitalis and of herpes labialis in nonimmunocompromised patients, there was no evidence of clinical benefit; there was some decrease in duration of viral shedding.

Diagnosis: Whereas cutaneous lesions associated with Herpes simplex infections are often characteristic, the finding of multinucleated giant cells in smears prepared from lesion exudate or scrapings may assist in the diagnosis.[1] Positive cultures for Herpes simplex virus offer a reliable means for confirmation of the diagnosis. In genital herpes, appropriate examinations should be performed to rule out other sexually transmitted diseases.

CONTRAINDICATIONS: Zovirax Ointment 5% is contraindicated for patients who develop hypersensitivity or chemical intolerance to the components of the formulation.

WARNINGS: Zovirax Ointment 5% is intended for cutaneous use only and should not be used in the eye.

PRECAUTIONS:

General: The recommended dosage, frequency of applications, and length of treatment should not be exceeded (see DOSAGE AND ADMINISTRATION). There exist no data which demonstrate that the use of Zovirax Ointment 5% will either prevent transmission of infection to other persons or prevent recurrent infections when applied in the absence of signs and symptoms. Zovirax Ointment 5% should not be used for the prevention of recurrent HSV infections. Although clinically significant viral resistance associated with the use of Zovirax Ointment 5% has not been observed, this possibility exists.

Drug Interactions: Clinical experience has identified no interactions resulting from topical or systemic administration of other drugs concomitantly with Zovirax Ointment 5%.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of 50, 150 and 450 mg/kg/day given by gavage. These studies showed no statistically significant difference in the incidence of benign and malignant tumors produced in drug-treated as compared to control animals, nor did acyclovir induce the occurrence of tumors earlier in drug-treated animals as compared to controls. In 2 *in vitro* cell transformation assays, used to provide preliminary assessment of potential oncogenicity in advance of these more definitive lifetime bioassays in rodents, conflicting results were obtained. Acyclovir was positive at the highest dose used in one system and the resulting morphologically transformed cells formed tumors when inoculated into immunosuppressed, syngeneic, weanling mice. Acyclovir was negative in another transformation system.

No chromosome damage was observed at maximum tolerated parenteral doses of 100 mg/kg acyclovir in rats or Chinese hamsters; higher doses of 500 and 1000 mg/kg were clastogenic in Chinese hamsters. In addition, no activity was found in a dominant lethal study in mice. In 9 of 11 microbial and mammalian cell assays, no evidence of mutagenicity was observed. In 2 mammalian cell assays (human lymphocytes and L5178Y mouse lymphoma cells *in vitro*), positive response for mutagenicity and chromosomal damage occurred, but only at concentrations at least 1000 times the plasma levels achieved in man following topical application.

Acyclovir does not impair fertility or reproduction in mice at oral doses up to 450 mg/kg/day or in rats at subcutaneous doses up to 25 mg/kg/day. In rabbits given a high dose of acyclovir (50 mg/kg/day, s.c.), there was a statistically significant decrease in implantation efficiency.

Pregnancy: ***Teratogenic Effects. Pregnancy Category C.*** Acyclovir has been known to cause a statistically significant decrease in implantation efficiency in rabbits, when given at subcutaneous doses providing mean plasma levels of drug 2.2 times those expected from use in patients with normal renal function.

Reproduction studies were negative for impairment of fertility or harm to the fetus in mice given oral doses, and in rats given subcutaneous doses providing mean plasma levels of drug 84 times and 4 times (respectively) greater than those expected from use in patients with normal renal function.

Acyclovir was not teratogenic after subcutaneous administration of up to 50 mg/kg/day during the period of organogenesis in rats and rabbits; doses up to 450 mg/kg given daily by gavage to mice were not teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Acyclovir should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Zovirax is administered to a nursing woman.

ADVERSE REACTIONS: Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of ointment. In the controlled clinical trials, mild pain (including transient burning and stinging) was reported by 103 (28.3%) of 364 patients treated with acyclovir and by 115 (31.1%) of 370 patients treated with placebo; treatment was discontinued in 2 of these patients. Other local reactions among acyclovir-treated patients included pruritus in 15 (4.1%), rash in 1 (0.3%) and vulvitis in 1 (0.3%). Among the placebo-treated patients, pruritus was reported by 17 (4.6%) and rash by 1 (0.3%).

In all studies, there was no significant difference between the drug and placebo group in the rate or type of reported adverse reactions nor were there any differences in abnormal clinical laboratory findings.

DOSAGE AND ADMINISTRATION: Apply sufficient quantity to adequately cover all lesions every 3 hours 6 times per day for 7 days. The dose size per application will vary depending upon the total lesion area but should approximate a one-half inch ribbon of ointment per 4 square inches of surface area. A finger cot or rubber glove should be used when applying Zovirax to prevent autoinoculation of other body sites and transmission of infection to other persons. **Therapy should be initiated as early as possible following onset of signs and symptoms.**

HOW SUPPLIED: Zovirax Ointment 5% is supplied in 15 g tubes (NDC 0081-0993-94). Each gram contains 50 mg acyclovir in a polyethylene glycol base. Store at 15°-25°C (59°-77°F) in a dry place.

REFERENCE: 1. Naib ZM et al. Cancer Res *33*:1452-1463, 1973.

U.S. Patent No. 4199574

83-ZOV-13

Wellcome
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Research Triangle Park
North Carolina 27709

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in pelvic infections*...

Artist's concept of the uterus
and adnexa as viewed through
a diagnostic laparoscope.
Pathology includes pelvic
cellulitis and pelvic
inflammatory disease.

*including endometritis, pelvic
cellulitis, and pelvic
inflammatory disease caused
by indicated organisms

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you've proved it to yourself

after 6 years the facts *still* favor Mefoxin® IV/IM

(Cefoxitin Sodium|MSD

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs. This product should be given with caution to penicillin-sensitive patients.

- **proven highly effective**
  Over 65 clinical studies document the effectiveness of MEFOXIN in the treatment of gynecological infections, including endometritis, pelvic cellulitis, and pelvic inflammatory disease.
- **proven stability**
  MEFOXIN has become the reference standard for stability against beta-lactamases which destroy other antibiotics.
- **proven utility**
  Current survey data show that obstetricians/gynecologists rely on MEFOXIN for the hospital treatment of pelvic infections more often than any other single injectable antibiotic.

Over 6 years of use in hospitals confirms MEFOXIN has a safety profile comparable to cephalothin. The most common adverse reactions have been local reactions following intravenous or intramuscular injection.

For a Brief Summary of Prescribing Information, please see following page.

MSD
MERC
SHARP
DOHM

Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

Indications and Usage: ***Treatment***—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Interference with Laboratory Tests—High concentrations (>100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST‖ reagent tablets.

Pregnancy—In women of childbearing potential, weigh anticipated benefit against possible risks.

Nursing Mothers—Cefoxitin is excreted in human milk in low concentrations.

Infants and Children—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions. *Gastrointestinal*—Symptoms of pseudomembranous colitis during or after treatment and, rarely, nausea and vomiting. *Blood*—Transient eosinophilia, leukopenia, neutropenia, hemolytic anemia, and thrombocytopenia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§*B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus*

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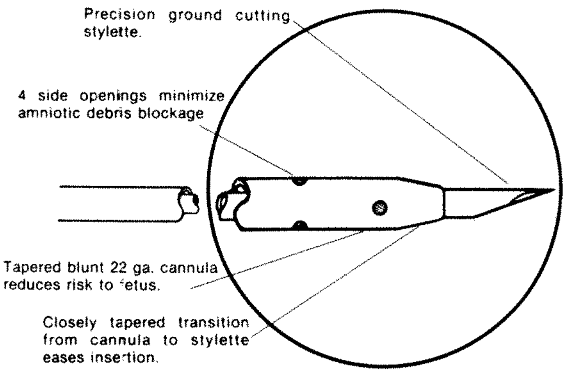
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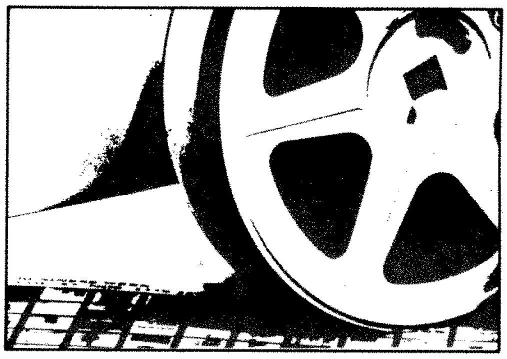

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American Journal of Obstetrics and Gynecology

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INFORMATION FOR AUTHORS

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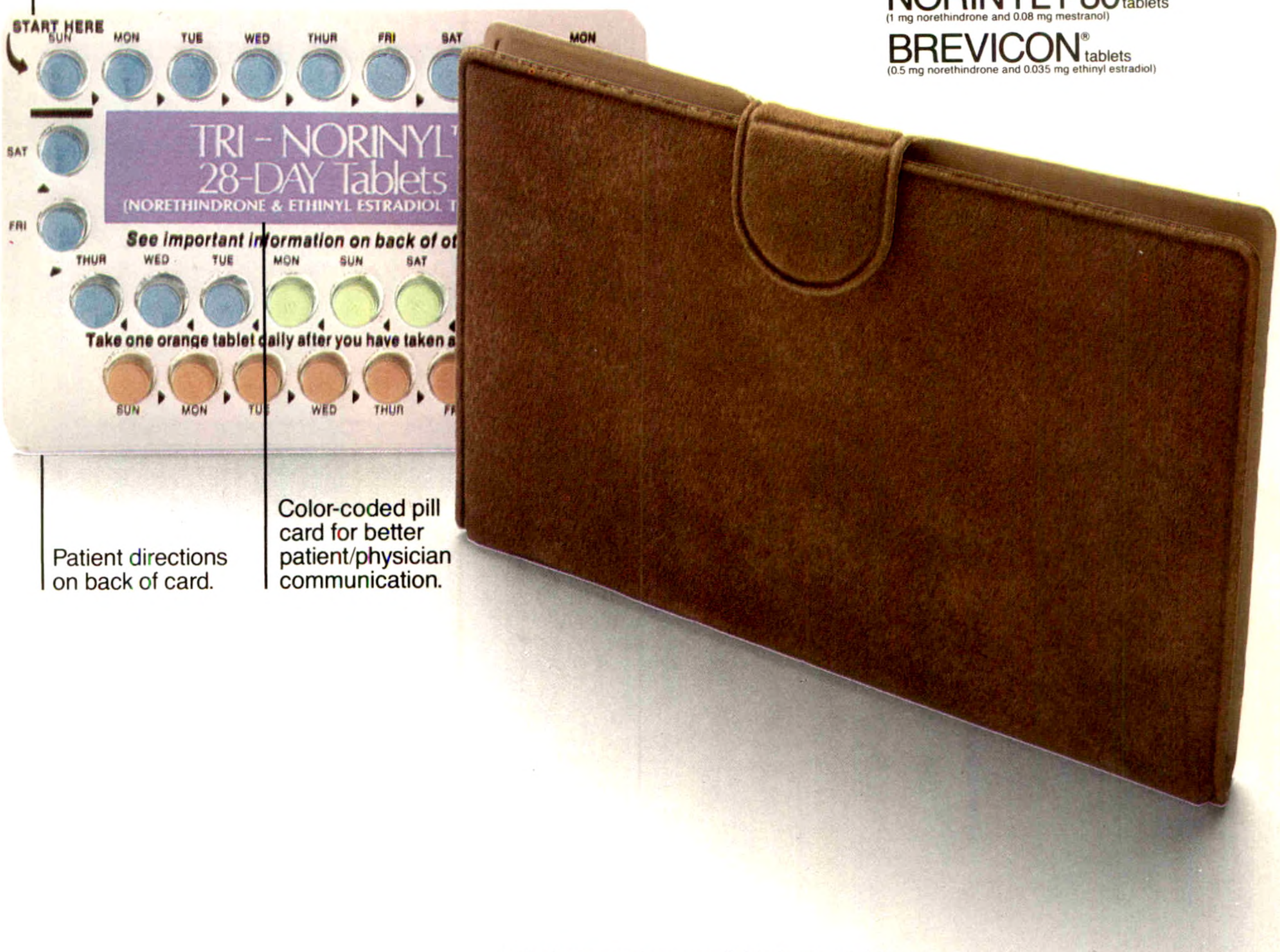
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Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

> **WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.**
>
> The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone. MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk.
B—Use associated with high risk.
C—Use associated with moderate risk.
D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert)

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TRANSACTIONS OF THE FORTIETH ANNUAL MEETING OF THE SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA

Differential symptom response to parenteral estrogen and/or androgen administration in the surgical menopause

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The investigation of estrogen and/or androgen administration on physical and psychological symptoms in the surgical menopause was carried out in a prospective, double-blind, crossover design. When patients who received either a combined estrogen-androgen drug or androgen alone were compared with those who received estrogen alone or placebo, energy level, well-being, and appetite were increased ($p < 0.01$). The androgen-containing preparations also induced lower somatic, psychological, and total scores on the menopausal index. Superior functioning in the androgen-treated groups occurred in association with higher plasma testosterone levels during the treatment phases ($p < 0.01$). These data suggest that reduced levels of circulating testosterone subsequent to bilateral oophorectomy may play an important rôle in the development of physical and psychological symptoms that are frequent sequelae of this surgical procedure. (AM J OBSTET GYNECOL 1985;151:153-60.)

Key words: Surgical menopause, estrogen and androgen, androgen parenteral therapy

The precipitate decline in plasma estrogen levels following bilateral oophorectomy in premenopausal women has been well documented.[1, 2] Less frequently acknowledged is the significant decrease in plasma testosterone levels subsequent to removal of both ovaries in this same population.[3] The observation that testosterone levels in ovarian vein blood are greater in early postmenopausal women than in reproductive-aged women[4] implies that patients who need to undergo bilateral oophorectomy are subsequently deprived of an important source of androgen production. Despite these known endocrine changes and the significant incidence of symptoms following total abdominal hysterectomy and bilateral salpingo-oophorectomy,[5] little evidence is available with regard to the functional role of androgen in the woman.

In order to evaluate possible differential effects of estrogen and androgen on symptoms in surgically menopausal women, a prospective, double-blind, crossover study was undertaken. The objectives of this investigation were twofold: (1) to independently assess the clinical response of somatic and psychological symptoms to exogenous estrogen and androgen administered singly as well as in combination and (2) to address the theoretical issues regarding physical and behavioral effects of the sex steroids in women.

Material and methods

Subjects. The participants in this study were premenopausal women who required a total abdominal hysterectomy and bilateral salpingo-oophorectomy for reasons other than malignant disease. They were re-

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			Treatment 1					*Treatment 2*		
Treatment groups	*Preoperative baseline*		*Month 1*	*Month 2*	*Month 3*	*Placebo, month 4*		*Month 5*	*Month 6*	*Month 7*
Estrogen-androgen combined		S U R G E R Y					C R O S S - O V E R			
Estrogen alone										
Androgen alone										
Placebo										
Hysterectomy control										

Fig. 1. Experimental design.

quired to be in a stable heterosexual relationship for at least 2 years and to have had no past history of psychological disturbances for which treatment had been obtained. A further requirement was that they be in a state of good general health with no known contraindications to hormone replacement therapy. Informed, signed consent was obtained from 43 women who met these criteria by means of a form approved by both a hospital and a university ethics committee. Ten women who needed to undergo total abdominal hysterectomy but in whom the ovaries were to be retained were recruited to serve as an additional control group. Between September, 1980, and February, 1983, each patient who was scheduled for total abdominal hysterectomy and bilateral salpingo-oophorectomy from the practice of the chief of the department of obstetrics and gynecology of a large university teaching hospital was referred to the study. Subjects were told that they would be participating in "an investigation of estrogen and androgen on physical and psychological functioning." They were further informed that there was a one-in-four chance that they would receive a preparation "with no active hormone" and that their medication might be changed during the course of the study. However, subjects had no way of knowing when that would occur or, indeed, if it would ever occur.

Procedure. The study was a five (groups)–by–four (time periods) repeated measures design. Before operation, patients were randomly assigned to either a combined estrogen-androgen group, an estrogen-alone group, an androgen-alone group, or a placebo group. The control hysterectomy group described previously constituted the fifth group. Thus there were two control groups in the design—one to control for the effects of an altered endocrine status consequent to bilateral salpingo-oophorectomy (placebo group) and the second to control for changes that may have been due to the surgical procedure itself (control hysterectomy group).

Following 1 month of baseline monitoring, patients underwent operation. Intramuscular injection of a hormonal preparation or placebo was administered monthly for the first 3 months (treatment 1). At the beginning of the fourth postoperative month, all patients received an injection of placebo. Women were then randomly crossed over to one of the four treatments they had not experienced during treatment 1 for an additional 3 months (treatment 2). Therefore, the entire experimental procedure encompassed a total of 8 months per patient. Fig. 1 contains a diagramatic representation of the experimental design.

Drug dosages. In order to ensure that patients in the hormone treatment groups would receive equal amounts of estrogen and androgen per dose, the molecular weights of the salts to which each of the estrogen and androgen preparations was bound were calculated. This sum was then subtracted from the weight of the total compound. With 1 ml of the combined estrogen-androgen drug used as the standard, equivalent doses of the sex steroids contained in the other preparations were computed. Accordingly, it was determined that 0.63 ml of the estrogen-alone drug and 0.48 ml of the androgen-alone drug contained amounts of estradiol and testosterone that were equivalent to amounts of these hormones in 1 ml of the combined preparation. While it is recognized that different esters have different durations of action, there did not appear to be any way to equate these except to calculate free estradiol. Moreover, it has been shown that other sex steroid depot preparations induce maximal plasma hormone levels 1 to 4 days following injection and that the half-life is 14 days.[6] The estrogen-androgen combined drug (Climacteron), 1 ml, contains testosterone enanthate benzilic acid hydrozone, 150.0 mg; estradiol dienanthate, 7.5 mg; and estradiol benzoate, 1.0 mg. The estrogen drug (Delestrogen), 1 ml, contains estradiol valerate, 10.0 mg. The androgen drug (Delatestryl), 1 ml, contains testosterone enanthate, 200.0 mg. The placebo consisted of sesame oil, 0.5 ml. After operation, drugs were given intramuscularly at 28-day intervals.

Plasma hormone levels. Venous blood samples for measurement of plasma levels of total estrogens (estradiol and estrone) and testosterone were obtained between 9 and 11 AM at four different times during the study. The first sample was taken approximately 1 month before operation; the second and fourth, 72 hours following the third injection in both treatment phases; and the third, on the last day of the placebo month that fell in between. The blood was immediately centrifuged and the plasma stored at −20° C. Total plasma estrogens were measured by radioimmunoassay with the use of Endocrine Sciences antiserum No. E-17-94. The extraction of protein before the addition of antiserum ensured that free hormone was being assayed. The assay was sensitive to the level of 12.5 pg/ml. Plasma testosterone levels were measured with the Covalent-Coat Radioimmunoassay Kit. The Testosterone (125 I) Assay detects the total unconjugated form of this steroid. All samples from each patient were measured twice in the same assay at the conclusion of the study. The control hysterectomy group underwent the identical experimental procedure except for the administration of monthly injections.

Material

Daily menopausal rating scale. Somatic and psychological symptoms were monitored daily by means of the daily menopausal rating scale. This instrument, devised and tested in a previous study,[7] was found to be a reliable and sensitive index of drug effects. Each symptom or behavior appears on an interval rating scale that has a range of 0 to 7. The poles of each scale have a verbal description of the symptom or behavior in question. Measures of energy level, well-being, appetite, and sleep quality reported here were monitored and quantified by means of the scale. Patients were given 30 questionnaires and 30 stamped, self-addressed envelopes at the beginning of each month and were instructed to fill in and mail a questionnaire daily. The 8-month duration of the study, therefore, yielded data on 240 days per patient and 12,720 patient days in all.

Menopausal index. The menopausal index, revised by Neugarten and Kraines,[8] was originally published by Blatt et al.[9] It lists 26 symptoms most often reported by clinicians and by women themselves as being typical or frequent complaints at menopause. Symptoms are categorized into three constellations—somatic (nine symptoms), psychosomatic (five symptoms), and psychological (12 symptoms) as seen in Table I. Because the scoring system that assigns differential weights to the various symptoms is not empirically based,[9] in this study, patients were required to rate the frequency and severity of each symptom on a bipolar rating scale that had a range of 0 to 7. The low end of the scale for each symptom was described as "almost never" while the extreme end stated "very often." Patients filled out the menopausal index at four points during the study immediately prior to venous blood collection.

Table I. Menopausal index

A. *Somatic symptoms*
  - Hot flushes
  - Cold sweats
  - Weight gain
  - Rheumatic pains
  - Cold hands and feet
  - Breast pains
  - Headaches
  - Numbness and tingling
  - Skin crawls

B. *Psychosomatic symptoms*
  - Feelings of suffocation
  - Blind spots before eyes
  - Pounding of the heart
  - Dizzy spells
  - Pressure or tightness in head or body

C. *Psychological symptoms*
  - Tired feelings
  - Irritable and nervous
  - Feel blue and depressed
  - Forgetfulness
  - Trouble sleeping
  - Inability to concentrate
  - Crying spells
  - Excitable
  - Attacks of panic
  - Loss of interest in most things
  - Feeling tense or wound-up
  - Worry needlessly

Statistical analyses. The hormonal and behavioral data were analyzed with the Computer Programs analysis of variance for groups × time blocks. For each item on the daily menopausal rating scale, the data analyzed were the mean of the preoperative baseline, the mean of the first 2 weeks of each treatment month, and the mean of the last 2 weeks of the intervening placebo month. In addition, analysis of covariance was also carried out in order to eliminate possible effects of initial group differences in baseline scores. Post hoc pairwise comparisons were performed with use of the Tukey test. Statistical significance reported here is based on results of the latter analyses.

Results

Subject characteristics. There were 12 patients in the combined estrogen-androgen group, 11 in the estrogen-alone group, 10 in the androgen-alone group, 10 in the placebo group, and 10 in the control hysterectomy group. Pathology reports confirmed that two patients had endometriosis; one was in the estrogen-alone group and the other in the placebo group. The rest of the sample (51 women) underwent operation for benign uterine fibroids. Five subjects who met the selection criteria and who were initially interviewed did

Table II. Mean plasma levels of total estrogen (estrone and estradiol) and testosterone at the four test times

	Estrogen-androgen	*Estrogen*	*Androgen*	*Placebo*	*Control*	*Significant differences**
Total estrogen (pg/ml), mean ± SEM						
Preoperative baseline	67.2 ± 10.4	49.4 ± 12.3	42.1 ± 11.2	69.4 ± 8.9	121.7 ± 7.4	$p < 0.01$†
Treatment 1	243.8 ± 12.4	301.9 ± 13.1	107.8 ± 8.4	11.2 ± 3.9	90.8 ± 9.4	$p < 0.01$‡
Placebo phase	7.8 ± 2.3	14.2 ± 3.3	9.5 ± 1.9	5.8 ± 3.7	78.9 ± 7.8	$p < 0.01$†
Treatment 2	236.2 ± 14.8	289.1 ± 15.4	98.3 ± 9.6	6.2 ± 2.3	74.7 ± 6.6	$p < 0.01$‡
Testosterone (ng/100 ml), mean ± SEM						
Preoperative baseline	81.1 ± 5.3	99.6 ± 4.7	71.9 ± 10.3	114.1 ± 9.6	90.8 ± 8.6	NS
Treatment 1	133.1 ± 12.4	89.4 ± 3.6	111.1 ± 6.6	87.8 ± 1.6	99.8 ± 3.6	$p < 0.01$§
Placebo phase	83.2 ± 4.9	65.3 ± 3.8	67.9 ± 2.5	66.2 ± 1.7	89.4 ± 4.7	$p < 0.01$‖
Treatment 2	129.2 ± 11.6	48.4 ± 2.6	115.3 ± 6.2	44.7 ± 3.2	88.8 ± 4.6	$p < 0.01$§

*Between-group post hoc Tukey tests.

†Control hysterectomy versus estrogen-androgen, estrogen alone, androgen alone, and placebo.

‡Estrogen alone versus androgen alone, placebo, and control hysterectomy.

§Estrogen-androgen versus estrogen alone, placebo, and control hysterectomy.

‖Control hysterectomy versus estrogen alone, androgen alone, and placebo.

not continue in the study. Two women decided to defer operation, one refused to comply with the experimental procedure after only 1 week, and a fourth was eliminated after the first postoperative month because she withdrew her acceptance of the random assignment procedure. The fifth patient was found to have an unsuspected carcinoma of the ovary at the time of operation and was thus eliminated from the study. All other subjects remained in the study through its entirety and each provided a complete set of observations.

Subject characteristics measured at baseline showed that patients in the control hysterectomy group were significantly younger than those in the four treatment groups (36.3 ± 2.2 versus 45.8 ± 3.3 years). This was an expected finding in view of the fact that age is the major criterion that guides the decision to remove or retain normal-appearing ovaries at the time of total abdominal hysterectomy undertaken for benign conditions. Subjects in all five groups were homogeneous with respect to occupational status and years of school. There was, however, a wide range in these variables within groups which increased the generalizability of the findings. All subjects had normal personality profiles[10] and satisfactory marital relationships.[11]

Total plasma estrogen and testosterone levels. Mean plasma levels of total estrogen (estrone and estradiol) and testosterone at the four test times appear in Table II. The interassay coefficient of variation of total estrogen was 9%. At baseline the younger control hysterectomy group had significantly higher total estrogen levels than the four treatment groups ($p < 0.01$), whose levels did not differ from each other. This was the only measure on which there was any significant difference between the five groups at baseline, which testifies to the success of the randomization procedure. At 72 hours following injection in both treatment phases, all groups had higher total estrogen levels than the placebo group ($p < 0.01$). Moreover, total estrogen levels of the estrogen-alone group were significantly higher during treatment than those of the androgen-alone and the control hysterectomy groups ($p < 0.01$) but were not different from those of the estrogen-androgen group. At the end of the placebo month, the control hysterectomy group with intact ovaries had significantly higher total estrogen levels than the four groups of women who had undergone oophorectomy. In these latter women the levels fell to the lower limit of assay detectability, which indicates that the estrogen administered during treatment 1 had been completely metabolized. In all cases, total estrogen levels of subjects who received the same drug did not differ when treatment 1 and treatment 2 values were compared.

The interassay coefficient of variation of plasma testosterone levels was 10%. During both treatment phases, the estrogen-androgen group had significantly higher testosterone levels than the estrogen-alone, placebo, and control hysterectomy groups ($p < 0.01$), but values did not differ significantly from those of the androgen-alone group. By 8 weeks after injection, testosterone values in steroid-treated groups also fell to levels below those of the control hysterectomy group and levels of the androgen-alone, estrogen-alone, and placebo groups were significantly lower than both their own baseline values and control hysterectomy group values ($p < 0.05$). Once again, testosterone levels of subjects who received the same drug did not differ when group means of the two treatment phases were compared. Moreover, between-group differences in both hormone levels at treatment 2 were the same as those found during treatment 1.

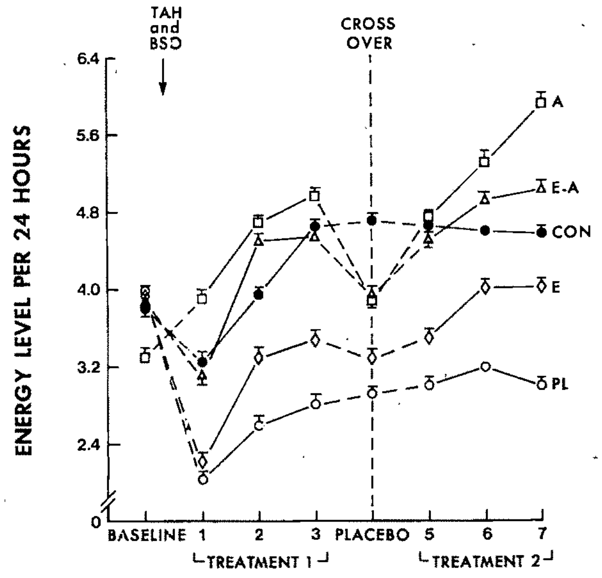

Fig. 2. Energy level for 24 hours. The treatment groups are: Estrogen-androgen group (*E-A*, △), estrogen-alone group (*E*, ◇), androgen-alone group (*A*, □), placebo group (*PL*, ○), and control hysterectomy group (CON, ●).

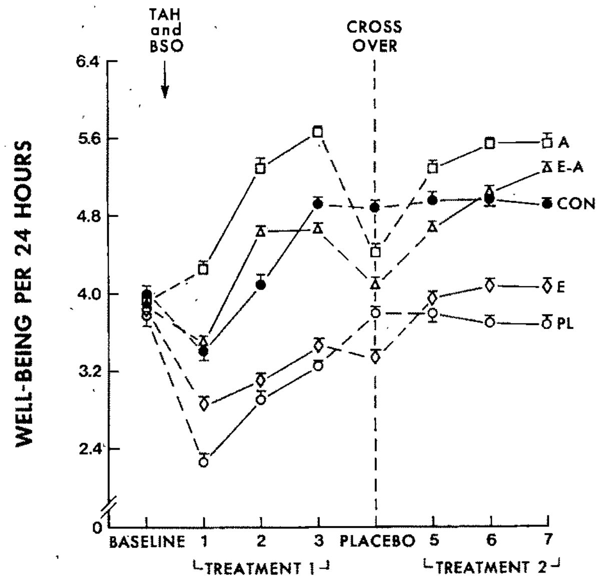

Fig. 3. Well-being per 24 hours. The treatment groups are as in Fig. 2.

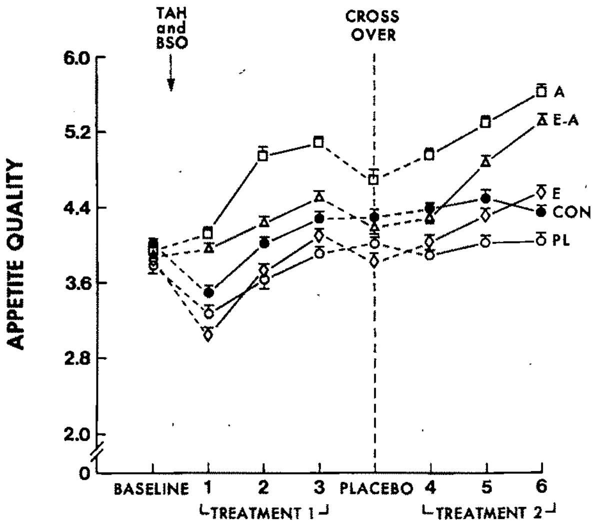

Fig. 4. Appetite quality. Treatment groups as in Fig. 2.

Energy level and well-being. Hormonal effects on these two measures, thought to reflect how patients were feeling in general, were very similar. In each of the three treatment months of both phases, women in the estrogen-alone and placebo groups reported significantly lower ratings of energy level and well-being than did the control hysterectomy group, with intact ovaries, and those who received either of the androgen-containing preparations ($p < 0.01$). Moreover, in treatment months 1, 3, 5, 6, and 7, estrogen-alone and placebo group scores were lower than those of the other three groups ($p < 0.01$). When hormones were withdrawn during the placebo month, the control hysterectomy group with intact ovaries attained higher scores on both measures than did all women who had undergone oophorectomy ($p < 0.01$) coincident with the higher plasma hormone levels at that time. It can be seen in Figs. 2 and 3 that there was a decrease in energy level and well-being from baseline to the first postoperative month in four groups ($p < 0.01$); only the patients who received androgen alone maintained energy level and well-being ratings in the immediate postoperative period. Furthermore, by the end of the seventh postoperative month, energy level and well-being scores of the control hysterectomy group and both groups that received androgen (estrogen-androgen and androgen alone) exceeded the preoperative baseline scores ($p < 0.01$) whereas scores of the estrogen-alone and placebo groups did not differ significantly from baseline values at that time.

Appetite. Analysis of daily ratings of appetite quality shown in Fig. 4 found that in each of the treatment months, scores of the estrogen-alone, placebo, and control hysterectomy groups were significantly lower than those of the androgen-alone group ($p < 0.01$) and lower than those of the estrogen-androgen group as well in months 1, 2, and 7 ($p < 0.01$). The lower estrogen-alone, placebo, and control hysterectomy group appetite ratings occurred in association with their lower testosterone levels in both treatment phases. During the placebo month, scores of the androgen-alone group decreased significantly compared to the treatment values ($p < 0.01$). By the third month in both treatment phases, appetite ratings were higher than preoperative baseline scores in only the estrogen-androgen and the androgen-alone groups ($p < 0.01$).

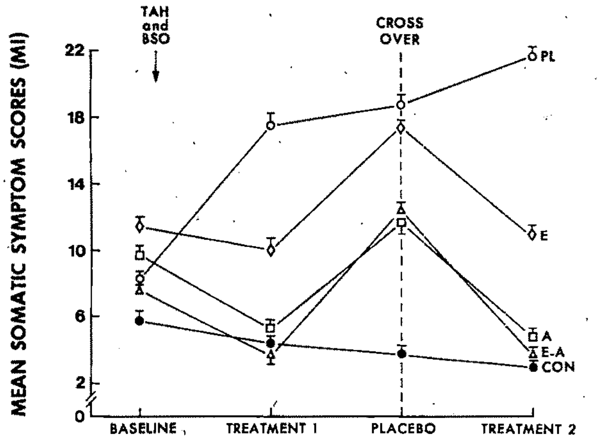

Fig. 5. Mean somatic symptom scores. Treatment groups as in Fig. 2.

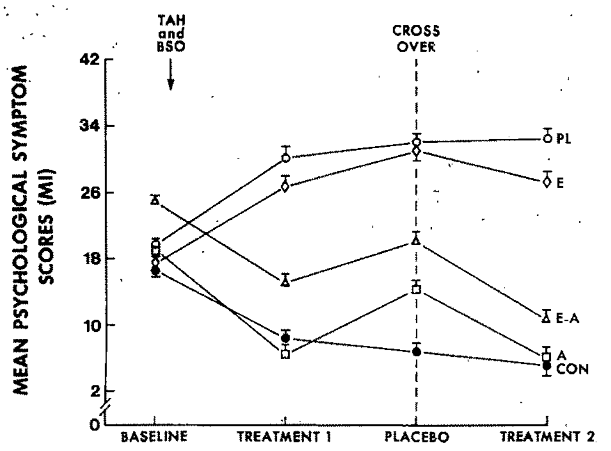

Fig. 6. Mean psychological symptom scores. Treatment groups as in Fig. 2.

Sleep quality. Analysis of daily ratings of sleep quality showed that there were no significant differences in any of the groups throughout the course of the investigation.

Menopausal index

Somatic symptoms. Lower scores on subtests of the menopausal index indicate a lower frequency and severity of symptoms. Fig. 5 shows that during both treatment phases somatic symptom scores of the estrogen-androgen, androgen-alone, and control hysterectomy groups were lower than those of the estrogen-alone and the placebo groups $(p < 0.01)$, whereas during the placebo month all women who had undergone oophorectomy reported higher scores than the control hysterectomy group $(p < 0.01)$. Furthermore, within-group analyses showed that all steroid-treated groups experienced a significant increase in scores during the placebo month compared to the treatment phase scores. However, scores of patients in the placebo group increased significantly and consistently after operation compared to the preoperative baseline values $(p < 0.01)$ whereas those of the control hysterectomy group remained stable at low levels throughout the course of the study.

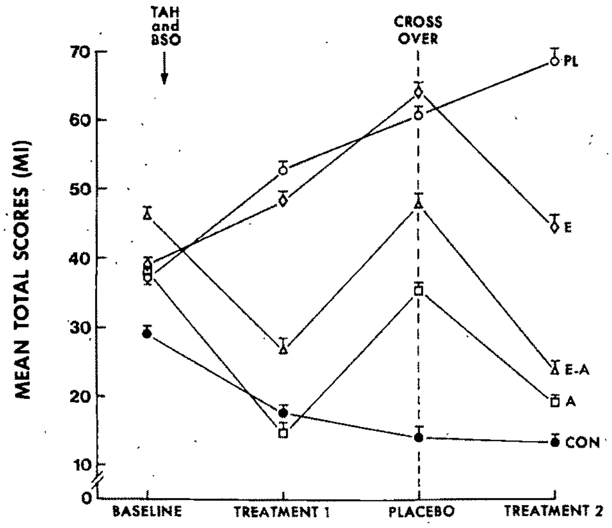

Fig. 7. Mean total scores. Treatment groups as in Fig. 2.

Psychosomatic symptoms. There were no significant changes in psychosomatic symptom scores in any of the groups across time.

Psychological symptoms. As seen in Fig. 6, psychological symptom scores of the estrogen-alone and placebo groups were significantly higher than those of the estrogen-androgen, androgen-alone, and control hysterectomy groups during both treatment phases $(p < 0.01)$. Moreover, during the intervening placebo month, scores of the control hysterectomy group were lower than those reported by the estrogen-androgen group $(p < 0.05)$ and by the estrogen-alone and placebo groups $(p < 0.01)$. Analysis of within-group changes across time found that baseline psychological symptom scores were lower than treatment phase scores in the estrogen-alone and placebo groups $(p < 0.01)$ whereas both androgen groups and the control hysterectomy group attained lower scores during the treatment phases compared to the baseline values $(p < 0.01)$.

Total scores. Composite scores of all items on the menopausal index appear in Fig. 7. The estrogen-androgen, androgen-alone, and control hysterectomy groups attained lower total scores during both treatment phases than the estrogen-alone and placebo groups $(p < 0.01)$. However, during the placebo month, total scores of the control hysterectomy group were lower than those of all women who had undergone bilateral salpingo-oophorectomy $(p < 0.01)$. Furthermore, in contrast to the lower scores attained by the estrogen-androgen and androgen-alone groups during treatment compared to the baseline and placebo phase scores ($p < 0.01$), the estrogen-alone and placebo groups reported higher total scores on this measure at all postoperative testing times compared to the preoperative values ($p < 0.01$).

Comment

Results of this study unequivocally demonstrated differential responses of physical and psychological symptoms to estrogen and/or androgen administration in surgically menopausal women. In view of the fact that patients in this investigation were generally healthy and functioning well preoperatively, these data also provide interesting information with regard to the incidence of symptoms in treated and untreated surgically menopausal patients.

The postoperative decrease in both total estrogen and testosterone levels in women who have undergone oophorectomy reflected in the placebo phase hormone assays is consistent with data reported previously.[1,3] The single exception was the absence of a statistically significant decline in the testosterone levels of the estrogen-androgen group by the end of the placebo month. It may be that endogenous testosterone levels did not decrease in this group subsequent to operation. However, an alternative hypothesis is that the half-life of the combined preparation is longer than the half-lives of the other drugs, and thus the dose administered had not been completely metabolized by the eighth week following the last injection. Studies on the pharmacodynamics of the combined preparation currently underway will provide a basis for resolving this issue.

Coincident with the observed increases in circulating testosterone levels in the estrogen-androgen and androgen-alone groups during treatment was enhancement of energy level, well-being, and appetite reported by these patients. The androgenic effect on these behaviors was likely a reflection of the anabolic[12] and energizing properties[13] of this steroid. The observation that androgen alone had a more profound effect on energy level, well-being, and appetite relative to the combined drug at several points during treatment can be explained by changes in sex hormone–binding globulin concentrations induced by the sex steroids. It is known that both estrogen and androgen bind to sex hormone–binding globulin in plasma and that only the free unbound portion has potential for biologic activity.[14] Because estrogen increases sex hormone–binding globulin levels and androgen reduces them,[15] it is possible to infer that the administration of androgen alone in the presence of low levels of endogenous estrogen resulted in a higher percentage of circulating free testosterone in the androgen-alone group compared to the estrogen-androgen group. The relative increase in free testosterone in the androgen-alone group may thus have accentuated the androgenic effects on behavior observed. This hypothesis merits testing by measurement of sex hormone–binding globulin subsequent to the administration of both of these drugs.

That exogenous estrogen alone was not more effective than placebo on energy level, well-being, and appetite was clearly demonstrated. Moreover, the finding that scores of the younger control hysterectomy group with intact ovaries were higher than their own baseline values by the end of the seventh postoperative month suggests that preoperative scores may have been depressed in all groups because of psychological factors related to impending operation. If this were the case, the stability in estrogen-alone and placebo group scores following operation may be indicative of an actual postoperative deterioration in their functioning. Studies that have reported increased well-being in postmenopausal women as a function of estrogen administration have been single-blind[16] or have used subjects undergoing a natural menopause[17] so that endogenous testosterone levels were likely to be unchanged. Other authors,[18, 19] however, have similarly failed to find a difference between effects of estrogen and placebo on well-being in the postmenopause. It is important to note that in this study increased energy level and well-being did not occur secondary to relief of somatic symptoms since it was found that androgen alone and placebo were equally ineffective in alleviating hot flushes in these same women.[20] Moreover, no side effects of exogenous androgen were either observed or reported. Specifically, there were no signs of virilization in the two androgen groups during either of the 3-month treatment phases.

The superior efficacy of the androgen-containing preparations on somatic, psychological, and total scores of the menopausal index may also be related to the anabolic and energizing properties of this sex steroid. Furthermore, the presence of androgen receptors in the hypothalamus, pituitary, and limbic system[21] allows for the possibility that this hormone may have been exerting its effects on behavior centrally. Additional evidence for a causal relation between these symptoms and circulating levels of sex steroids derives first from the observation that, when hormones were withdrawn during the placebo month, scores of all oophorectomized subjects on the three subscales of the menopausal index increased compared to the treatment phase scores. Second, scores of the placebo group increased consistently and significantly during the postoperative course compared to preoperative values in association with a decrease in circulating total estrogen and testosterone levels. In contrast, scores of the intact control hysterectomy group on the menopausal index maintained stability throughout the study concomitant with stability in sex steroid levels across time.

The consistent clinical superiority of the androgen-containing drugs in this study strongly implies that reduced levels of circulating testosterone may indeed play a critical functional role in the development of a myriad of disturbances that often arise in premenopausal women subsequent to bilateral salpingo-oophorectomy. The clinical implication of these findings is that administration of both sex steroids to this population may militate against symptoms that are a consequence of the sudden and drastically altered endocrine status induced after operation. These results need to be confirmed both by replication of this study and by investigation of the long-term effects of combined hormone replacement therapy in surgically menopausal women.

These data serve as well to point out a methodologic issue in menopause research. It was noted that the effect of estrogen on hot flushes, the cardinal menopausal symptom, was obscured when it was grouped together with other symptoms, as is the case in the menopausal index. This finding underlines the importance of investigating menopausal symptoms independently until we derive an empirical basis for clustering them.

In summary, results of this study showed that premenopausal women who received androgen alone or a combined estrogen-androgen drug following total abdominal hysterectomy and bilateral salpingo-oophorectomy reported fewer somatic and psychological symptoms than those who received estrogen alone or placebo. Furthermore, the androgen-containing preparations induced levels of functioning that were similar to those of younger women with intact ovaries. Bearing in mind that neither androgen alone nor placebo alleviated hot flushes,[20] the necessity for administering estrogen as well seems clear. These data provide strong support for the conclusion that a combined estrogen-androgen regimen may serve to enhance the quality of life of women who have experienced a surgical menopause.

Finally, it was observed in this study that menopausal symptoms are dissociable, under different hormonal control, and some, such as sleep quality and psychosomatic symptoms, independent of circulating levels of the sex steroids. In order to further investigate the specificity of hormone-behavior relationships, future research in this area ought to focus on the response of individual symptoms to the administration of each of the sex steroids as well as to their combined effect.

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Chorionic villi sampling: Clinical experience, immediate complications, and patient attitudes

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To develop chorionic villi sampling as a procedure for prenatal diagnosis, a pilot study was undertaken to perfect the obstetric and laboratory techniques, to evaluate our success with the procedure in continuing pregnancies, and to assess the attitudes of potential users of the procedure. Women about to have elective first-trimester abortions for nongenetic reasons were enrolled in the first phase of the study. Of the patients with a positive pregnancy test, 12.4% were found to have a nonviable pregnancy on ultrasound examination. Samples adequate for cytogenetic analysis were obtained in 130 of the 155 remaining cases, and the success rate was 93% in the 100 most recent cases. Direct cytogenetic analysis was undertaken in those cases successfully sampled, and karyotypes could be prepared in 97%. Immediate complications occurred in 5% of the pregnancies. Eight women at risk of bearing a child with a genetic defect had diagnostic chorionic villi sampling. Cytogenetic analysis was performed successfully on all of them. One had an induced abortion following the procedure because of the fetal diagnosis (a male with a 50% risk of Duchenne's muscular dystrophy). The other pregnancies are continuing uneventfully at 22 to 35 weeks' gestation. Finally, from preliminary analysis of our survey of potential users it appears that women 35 years old or over would prefer chorionic villi sampling to amniocentesis if the risks of the sampling were known to be low. (AM J OBSTET GYNECOL 1985;151:161-6.)

Key words: Chorionic villi sampling, clinical experience, cytogenetics, prenatal diagnosis

Prevention of genetic disease by prenatal diagnosis has become an increasingly important component of antenatal medicine.[1] It is now well established that, in the absence of prenatal diagnosis, women with a known high risk of having a severely affected baby avoid pregnancy.[2] If pregnancy does occur, they seek abortion.[3] On the other hand, since it has become possible to diagnose genetic disease in the fetus, the fertility rate among high-risk couples has increased and the induced abortion rate has decreased.[2]

Until recently, couples who elected to undergo prenatal diagnosis have had to wait until 16 weeks' gestation for amniocentesis and another 2 to 4 weeks for the results of biochemical and/or cytogenetic studies. The long waiting period can produce considerable parental distress. Furthermore, second-trimester abortion is associated with significantly higher risks of maternal morbidity and mortality than is a first-trimester procedure.[4] Thus a number of investigators have attempted to develop a safe and reliable method of first-trimester prenatal diagnosis.[5-9] The recent availability of high-resolution ultrasound, highly sophisticated technology for DNA analysis, and cytogenetic analysis of embryonic tissue have made chorionic villi sampling a timely development.

Ward et al.[10] have shown that under ultrasound guidance chorionic villi can be successfully aspirated from the developing trophoblast with a flexible catheter inserted through the cervical canal (Fig. 1). This tissue has been shown to be suitable for cytogenetic,[11] biochemical,[12, 13] and DNA analyses.[14]

Our investigation of chorionic villi sampling has had three primary objectives: to perfect the technique and assess our ability to obtain a sample and make a diagnosis, to evaluate our success in applying the procedure in diagnostic cases, and to explore the attitudes of women who might be eligible for this procedure. In addition, we were interested in determining whether

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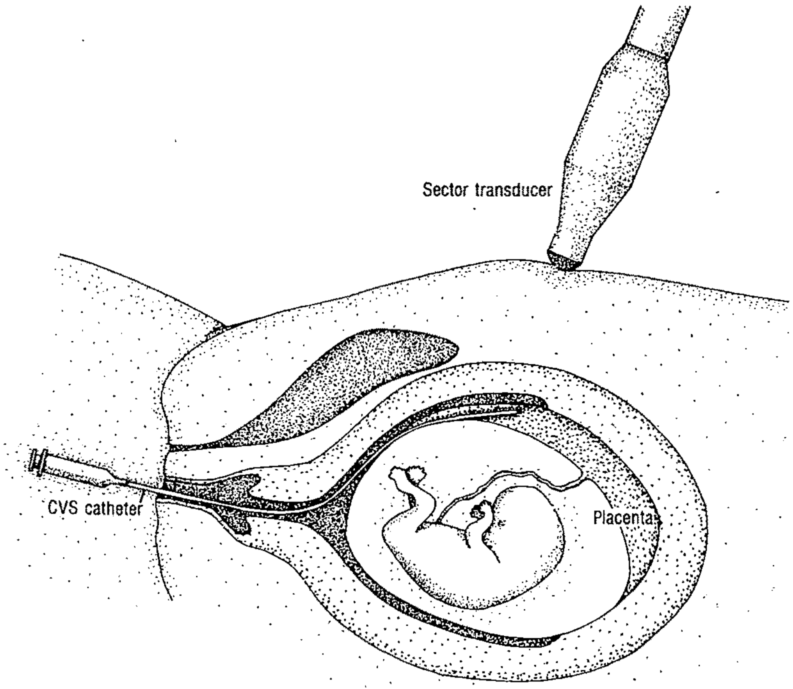

Fig. 1. Diagrammatic illustration of the ultrasonically guided transcervical approach to chorionic villi sampling.

maternal serum α-fetoprotein values increase following chorionic villi sampling, since such changes might interfere with the interpretation of second-trimester values obtained to diagnose neural tube defects.

Material and methods

To perfect the technique of chorionic villi sampling, we asked women scheduled for elective termination of pregnancy at gestational ages ranging from $6\frac{2}{7}$ to 15 weeks to agree to the procedure for research purposes. The study was approved by ethics committees at the hospitals involved. Chorionic villi sampling was performed transcervically under real-time ultrasonic guidance in cases with an ultrasonically proved viable fetus. The tip of a 1.5 mm in diameter by 21 cm long catheter with malleable stylet (Portex, Inc., Wilmington, Massachusetts; Vas-Cath Ltd., Mississauga, Ontario) was inserted into the placenta, the stylet withdrawn, and villus material removed by repeated aspiration. The catheter tip was continuously moved within the placenta in a short oscillating longitudinal direction for about 15 seconds during each aspiration to help dislodge tissue. The sample was washed into Hank's solution containing 5 units of heparin per milliliter of solution. Single fragments showing typical villous morphology were dissected from the sample with sterile technique and washed twice in the Hank's solution. They were then transferred into culture medium containing 10% fetal calf serum for transport to the Montreal Children's Hospital for cytogenetic analysis. For each case a record was kept of maternal and gestational ages, gravidity, parity, complications during this and other pregnancies, number of catheter insertions, estimated weight of tissue removed, presence of fetal heart beat before and after the procedure, and complications during the procedure. To be considered successful, chorionic villi sampling had to provide 10 mg of tissue, since this was the amount required for the application of several cytogenetic techniques required to determine the fetal karyotype. In 36 cases maternal blood was obtained immediately before and after the procedure for α-fetoprotein assay.

To assess the technique clinically in women with fetuses at risk for a genetic disorder, chorionic villi sampling was also performed on eight patients who intended to continue the pregnancy if it were found to be unaffected. We limited diagnostic chorionic villi sampling to women at high risk of having an affected baby or to those who expressed extreme anxiety about amniocentesis because of past experience. Their ultrasonically determined gestational ages ranged from $8\frac{0}{7}$ to $11\frac{1}{7}$ weeks. Data identical to that recorded for the pregnancy termination cases were obtained for each of these women.

Finally, to investigate women's attitudes to chorionic villi sampling in the face of the currently unknown risks of the procedure, all women scheduled for amniocentesis between December 1, 1983, and March 31, 1984,

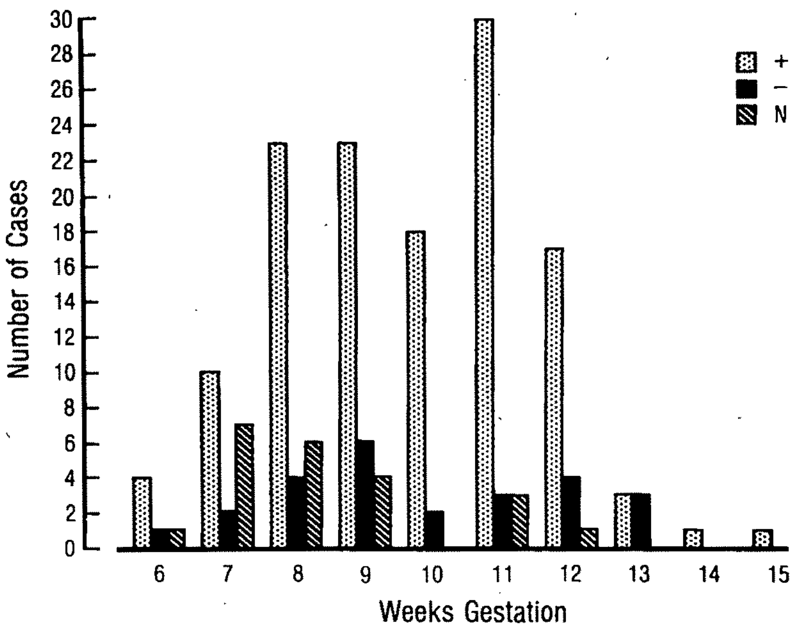

Fig. 2. The number of cases at gestational ages 6 through 15 weeks in which chorionic villi sampling was successful *(dotted bar)* or unsuccessful *(solid bar)* or those in which there was a blighted ovum *(striped bar)*.

solely because they would be ≥35 years at the time of delivery were surveyed by questionnaire about their preferences for chorionic villi sampling or amniocentesis. The questionnaire contained detailed descriptive material about chorionic villi sampling and amniocentesis (such as when in pregnancy each is performed, how each is done, the length of time between procedure and results, the risks—known and unknown—of spontaneous abortion attributable to each procedure, and the method of induced abortion should the woman choose to terminate the pregnancy). A graph and a table were also included so that each woman could determine her own age-specific risk of having a child with trisomy 21 (Down syndrome). The women were asked to read this material and then to rate the importance of the described differences between chorionic villi sampling and amniocentesis. They were also asked to indicate which of the two they preferred on the basis of each individual difference between procedures as well as when taking all the factors jointly into consideration.

Results

Studies in therapeutic abortion cases. Between June 1, 1983, and April 30, 1984, 177 women with positive pregnancy tests, who were undergoing therapeutic abortion for nongenetic indications, consented to participate in the study. Ultrasonic examination immediately prior to the procedure revealed either a blighted ovum or a nonviable fetus in 22 cases (12.4%). In the remaining 155 cases we obtained ≥10 mg of villi (as estimated by visual comparison of the sample with photographs of weighed quantities) in 130, for an overall success rate of 84%. A ≥10 mg sample was obtained with only one aspiration in 45% of cases, after two aspirations in 25%, and after three in 30%. Among the most recent 100 cases in which sampling was attempted, the success rate has been 93%. In six cases an abruptio placentae occurred (3.8%), and in two cases the amniotic sac was ruptured (1.3%). In the last 100 cases the incidence of abruptio placentae and sac rupture were 5% and 0%, respectively. The number of successful samplings and the frequency with which nonviable fetuses were found according to gestational age are given in Fig. 2.

Cytogenetic preparations adequate for karyotyping were produced routinely from villous material within 1 day and more recently within 2 hours. Using these rapid techniques, we have been able to prepare a fetal karyotype in 88% of the 97 specimens submitted to the laboratory since the study started. By means of the same methods, a success rate of 97% has been achieved on the last 55 specimens submitted. These data are in close agreement with data published by others.[15] A detailed account of the cytogenetic methodology and results will be presented elsewhere. It should be noted, however, that banding quality following direct preparations is not as good as that obtainable routinely on cultured amniotic fluid cells. Thus diagnosis of structural aberrations may be difficult with the direct technique. However, short-term culture techniques can yield reasonably good-quality banded karyotypes in 2 days, and

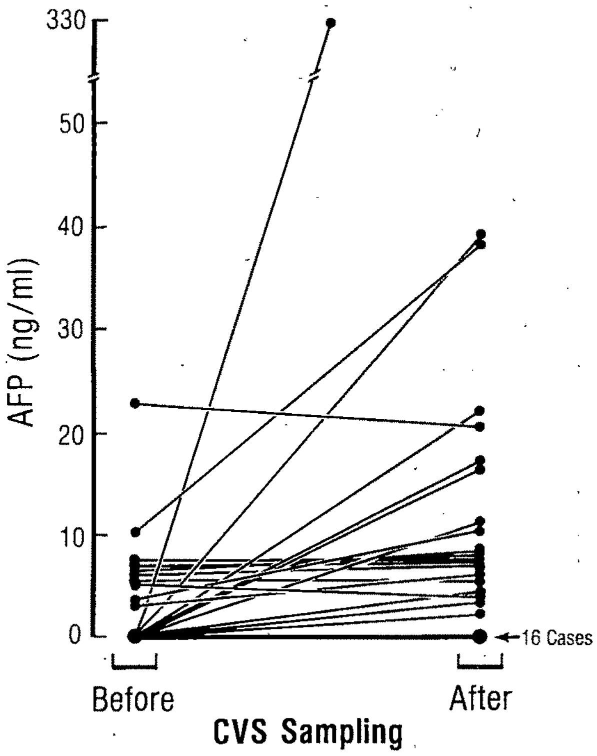

Fig. 3. Maternal serum α-fetoprotein *(AFP)* before and after chorionic villi sampling *(CVS)* in 36 pregnancy termination cases.

we anticipate that in the near future those preparations will be as good as the ones obtained after long-term culture.

Continuing pregnancy. Eight women consented to diagnostic chorionic villi sampling. The indications for prenatal diagnosis in these cases were carrier status for Duchenne's muscular dystrophy (1 case), previous child with trisomy 21 or 18 (2 cases), child at risk for sickle cell anemia (1 case), and maternal age (4 cases). A cytogenetic analysis was performed successfully in all of them. The Duchenne carrier was found to have a male fetus, and the couple decided to abort the pregnancy because of its 50% risk. The other seven pregnancies are continuing uneventfully at gestational ages ranging from 22 to 35 weeks. A detailed ultrasound examination and maternal serum α-fetoprotein analyses were performed for each continuing pregnancy at 16 to 17 weeks' gestation to rule out a neural tube defect.

Attitudes to chorionic villi sampling. From a preliminary analysis of 134 completed questionnaires returned to the McGill Prenatal Diagnosis Programme, we found that, given current knowledge of the techniques, women scheduled for routine amniocentesis were almost equally divided in their preferences for chorionic villi sampling and amniocentesis: 46% versus 47% respectively, with 7% indicating no preference. However, if chorionic villi sampling were found to be only as risky as amniocentesis, 82% of the women said they would chose chorionic villi sampling, whereas if the risk of miscarriage following chorionic villi sampling were to be 5% higher than that following amniocentesis, 71% of the women chose amniocentesis. In both hypothetical situations, 6% had no preference.

Comment

Chorionic villi sampling appears to offer a number of advantages to couples seeking prenatal diagnosis provided that it can be shown to be successful in supplying fetal material for analysis and that it is both safe and accurate. Our rate of success in obtaining villus material has risen steadily as we have gained experience. On the one hand, we recognize that the pressure to minimize operating room time when sampling pregnancy termination cases is an inducement to abandon the procedure if one has not obtained a sample after three attempts, thus decreasing the success rate. On the other hand, persistence may improve the rate of sampling success while placing the pregnancy in jeopardy in cases that are to continue if the fetus is unaffected. It may be preferable to postpone sampling for a week after three failed attempts.

Although the numbers are small, the data in Fig. 2 indicate that sampling is less likely to be successful early and late in the first trimester. Visualization of the thickest area of chorion and the fetal heart were difficult in the sixth and seventh weeks, whereas reaching the placenta if it was located high in the uterus became increasingly difficult as the gestational age progressed beyond 12 weeks. This later problem is partially related to the length of catheters available, and the suppliers have introduced new models for such cases. In our most recent 150 cases, we have had no failures to obtain a sample at $12\frac{0}{7}$ to $12\frac{6}{7}$ weeks in 22 attempts. Therefore from the present data and Fig. 2, it appears that 9 to 12 weeks is the ideal time to perform chorionic villi sampling to obtain a sample successfully.

We have found that moving the catheter in a longitudinal back-and-forth motion during aspiration increases the number of villi obtained. So far, this technique does not appear to be associated with amniotic sac rupture or the production of abruptio placentae. These complications tend to occur during insertion of the catheter and placement of the tip in the desired placental area. The significance of abruptio placentae at this stage of pregnancy is still unknown. It may precipitate miscarriage or intrauterine growth retardation.

Data from a number of centers performing chorionic villi sampling are being collected to obtain preliminary information about the overall loss rates following the procedure and their correlations with procedure-related trauma.* It is important to point out that the miscarriage rate following chorionic villi sampling cannot be compared per se with the miscarriage rate following amniocentesis: the background spontaneous miscarriage rate is expected to be higher during early gestation when chorionic villi sampling is performed than at 16 weeks when amniocentesis is performed.[16] Rather, what is important is the *increased* miscarriage rate following chorionic villi sampling compared with the rate among fetuses ultrasonically proved viable at the time when chorionic villi sampling would have been performed but not subjected to the sampling. To obtain this information, a clinical trial is essential before diagnostic chorionic villi sampling is so widely available that it becomes ethically difficult to conduct a trial, such as has been the case with proposed trials of vitamin supplementation for neural tube defects.[17-19] We have already begun a randomized clinical trial of chorionic villi sampling and plans for other trials are well under way in Canada and elsewhere.

It is also important to determine the accuracy of the procedure. Although it is possible for maternal cell contamination to confound the cytogenetic results,[20] we have found that this problem is limited to results obtained from cultured cells (one of five males, 95% confidence limits = 0.5% to 71.6%) and does not occur in direct cytogenetic preparations (0 of 40 males, 95% confidence limits = 0% to 8.9%). This problem should be reduced further with improvement in technical procedures and the use of chromosomal heteromorphisms as it has been for amniotic fluid cells. Another possible source of error involves cytogenetic discrepancy between trophoblastic and fetal tissue.[21] Although the frequency of this observation around 10 weeks' gestation has not been clearly established, preliminary data suggest that a karyotypic discrepancy between these two tissues is uncommon, since out of 147 pairs of tissues investigated, none were discordant, even in the presence of mosaicism (95% confidence limits = 0% to 2.4%).[22]

The data in Fig. 3 indicate that maternal serum α-fetoprotein rises in 33% (12/36) of cases following chorionic villi sampling. Neither the cause nor the significance of this rise is clear to us. In our small series there was no relationship between serum α-fetoprotein increase and number of catheter insertions or amount of villous material aspirated. However, we are collecting data on serum α-fetoprotein before and after chorionic villi sampling in continuing pregnancies to determine whether a large increase is associated with subsequent miscarriage and is thus a predictor of this complication. We are also concerned that the half-life of a high-serum α-fetoprotein may be long enough to complicate detection of neural tube defects in the second trimester. Whereas most such defects should be detectable by a thorough ultrasound examination at 17 weeks' gestation, when there is no chorionic villi sampling, a raised serum α-fetoprotein would be an indication that amniocentesis should be performed; however, a persistently high serum α-fetoprotein level would confound interpretation of results and might contribute to a neural tube defect being missed.

In assessing the usefulness of a new medical procedure, it is important to know whether it is acceptable to the members of the population in which it will be applied. We have begun, therefore, to measure women's attitudes toward chorionic villi sampling as a method of prenatal diagnosis. Preliminary analysis of questionnaire responses suggests that women over 35 years base their preference for chorionic villi sampling or for amniocentesis primarily on two factors: the gestational age at which the procedure is carried out and the risk that the procedure may cause a spontaneous abortion. If, following randomized clinical trials, chorionic villi sampling were to be shown to be only as risky as amniocentesis, our preliminary data suggest there would be a substantial preference for it. Yet, it is equally interesting that if the additional risk attributable to chorionic villi sampling were but 5% higher than that associated with amniocentesis, it might no longer be the method of choice for the vast majority. These findings suggest that knowledge of the risks of chorionic villi sampling, and determination whether these are <5% over background, will be important in estimating ultimate acceptability of the procedure for women over 35 years seeking prenatal diagnosis.

In conclusion, we anticipate that if chorionic villi sampling is eventually shown to be safe, accurate, reliable, and cost effective, it may offer an attractive alternative to amniocentesis, especially for women who would not consider a second-trimester abortion. The extension of prenatal diagnosis to additional numbers of women at risk for having a child with a genetic disorder will be a welcome advance.

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Screening for sexual problems through a simple questionnaire

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Screening for sexual problems should be part of the medical assessment, but there is controversy on the best method to use. This study compared the yield of a simple questionnaire with that of a more detailed interview. Ninety-eight sexually active women (out of a total of 110) admitted on an elective basis to general gynecology wards were studied. Forty-six patients presented with a sexual complaint and 33 (33%) of these requested medical attention for the problem. In all cases, the simple questionnaire was as effective as the detailed inquiry in detecting a sexual problem. It is concluded that a simple questionnaire can be used effectively by all physicians as a screening test for sexual dysfunction with regard to heterosexual coital activity. Further research is required to confirm this hypothesis in other conditions of medical practice. (AM J OBSTET GYNECOL 1985;151:166-8.)

Key words: Sexuality, dysfunctions, interview, questionnaire

Health professionals are becoming increasingly aware of the importance of sexual health to individuals and couples. While most sources agree on the need to incorporate questions on sexual functioning as part of the medical assessment,[1] there is still considerable debate on the appropriate format to do so. This study compares the yield of a very simple questionnaire easily incorporated into a routine history with that of a more thorough sexual inquiry as a method of screening for sexual dysfunctions in women. Our review of the literature indicates that this is the first such study in which this format was used.

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Method

Ninety-eight sexually active women (out of a total of 110) admitted on an elective basis to general gynecology wards were studied. The study took place in two university hospitals located in a cosmopolitan urban center. The demographic characteristics of the population studied are presented in Table I.

The simple questionnaire consisted of three (3) questions: (1) Are you sexually active? (2) Are there any problems? (3) Do you have any pain with intercourse?

The more detailed interview included the above three questions and specific inquiry about: (1) difficulty with lubrication, (2) pain at penetration, (3) deep dyspareunia, (4) difficulties in achieving orgasm, (5) degree of satisfaction with sexual activity, (6) number of partners, and (7) any other concern.

Patients were not randomly allocated to either group of the study. Allocation was based on unrelated factors, such as patient flow and proposed surgical procedure.

Group 1. This group consisted of 53 patients, 50 of whom were sexually active. During the admission medical history and physical examination, I administered both questionnaires in sequence. The interview was conducted in private with ample time available for discussion of problems.

Group 2. This group comprised 57 patients, 48 of whom were sexually active. During the admission history and physical examination, patients were administered the first questionnaire only by either a senior medical student or an intern. Neither students nor interns had received any particular training in sexual history taking. The interview was conducted as per the usual ward routine, in four-bed rooms or in the examination room. I then saw the patients and administered the second questionnaire during a thorough review of the patient's presenting symptoms and gynecologic status. This interview was conducted in private with ample time available for discussion of problems.

Appropriate referral was offered to all patients who requested medical attention for their sexual problem.

Results

Group 1. Of the 50 sexually active patients, 26 complained of some form of sexual problem and 18 of these actually requested medical attention for the problem. The diagnostic categories and frequency of occurrence are presented in Table II.

The simple questionnaire detected all cases in which a sexual complaint was elicited.

Group 2. Of the 48 sexually active patients, 20 complained of some form of sexual problem and 15 of these actually requested medical attention for the problem. The diagnostic categories and frequency of occurrence are presented in Table II.

Table I. Demographic characteristics of the study population

Characteristic	*No.*
Age (yr)	
(Mean)	37.6 ± 1.5
(Min./max.)	22/79
Ethnic origin	
Anglo-American	38
French	39
Italian	13
Asiatic	8
Other	12
Religion	
Roman Catholic	68
Protestant	24
Hindu	7
Other	11
Marital status	
Single	10
Married	75
Divorced	18
Widowed	7
Obstetric history	
Gravidity (mean)	2.5 ± 0.15
Parity (mean)	2.1 ± 0.14
Contraceptive method used	
None/not applicable	63
Oral contraceptive	17
Tubal ligation	14
Intrauterine contraceptive device	8
Other	8

The simple questionnaire as administered by the student or intern again detected all cases in which a sexual complaint was elicited.

Comment

Reports on the prevalence of sexual dysfunctions vary from a low of 5%[2] to a high of 50%.[3] Most reports estimate the prevalence at about 25% in the general population.[4] Sexual complaints may reflect physical or psychological abnormality; they may represent a disease entity in themselves or reflect an underlying disturbance of conflict.

Along with psychiatrists and family physicians, obstetricians and gynecologists are the group of physicians most likely to encounter patients presenting with these problems.[5] It is now well accepted that a sexual history should be part of the routine medical assessment.[1] However, most sources emphasize how awkward both physician and patient may be in discussing this subject. These same sources advocate the inclusion of the sexual assessment in the review of systems but in fact continue to set it apart by recommending the use of various "introductory" statements.[6]

The use of a screening questionnaire has been suggested. One source claims that up to 95% of sexual problems can be detected in this fashion.[7] The scope of the questionnaire is still debated.[6-9] One common criticism of the busy practitioner is that these question-

Table II. Preliminary diagnoses and frequency of occurrence: combined data

Diagnosis	*Complaint* (No. of patients)*	*Dysfunction† (No. of patients)*
Arousal disorder	3	4
Vaginismus	0	8
Deep dyspareunia	7	13
Sexual desire disorder	1	6
Other	2	2

*Complaint is defined as a problem reported by the patient for which no medical attention is requested by the patient.

†Dysfunction is defined as a problem for which the patient requests medical attention in order to correct the problem.

naires are time consuming and demand ease in dealing with the issues. Many find it difficult to question the patient openly about difficulties with lubrication or orgasm.

With these concerns in mind, R. A. H. Kinch, M.D. (written communication) and J. Lamont, M.D. (written communication) have devised the simple questionnaire used in this experiment. By asking these simple questions the physician implies that he values the importance of sexuality and is available to discuss sexual problems.

The aim of this study was to compare the yield of this simple screening questionnaire to that of a more detailed review of sexual function in women. Our attention was focused on problems relating to heterosexual coital activity.

In group 1, we studied the yield under optimal circumstances (privacy, time, experienced interviewer). Under these conditions, the simple questionnaire allowed us to detect all cases in which a sexual problem was reported. The questionnaire was therefore as effective as the detailed review of sexual function. In group 2, the simple questionnaire was integrated in the routine ward admission procedures without any particular attention. The detailed review of sexual function was then administered under optimal conditions. Once more, the yield of the simple questionnaire was equal to that of the more detailed interview.

These findings are quite remarkable. On the basis of current literature, the more thorough review of sexual function was expected to have a higher yield. In addition, one would expect a "priming" phenomenon to occur when the simple questionnaire precedes the more thorough review of sexual function. Our results challenge these views.

There is still no universally recognized instrument for the detection of sexual dysfunctions. The reportedly more sensitive instruments require experienced professionals and much time. In view of the present findings however, the next step would be to compare the yield of the simple questionnaire to that of more advanced techniques.

The prevalence of sexual problems and dysfunctions was comparable to that previously reported in the literature. We believe it to reflect the true prevalence of dysfunctions in our study population.

The findings in this experiment support the hypothesis that patients will report sexual problems to a receptive physician who displays a willingness to discuss the subject. Ease with the subject matter may be less important than has been estimated up to now.

Many clinicians feel inadequate in the area of sexual therapy. They state that they lack the appropriate training to initiate brief or extended therapy. For this reason, they feel it is best to leave out inquiries about sexual functioning. While they must be commended for realizing their limitations, we cannot support this attitude. It is well acknowleged that many sexual concerns can be dealt with by simple reassurance. The PLISSIT model, developed by Annon,[10] supports this concept. More important, however, is the fact that there are resources available in most areas for advanced sexual counseling. In addition, the physician has a responsibility to rule out any physical abnormality as the cause of the sexual problem.

We advocate the use of the simple questionnaire in the same way as any other screening test is used. One would not withhold performing a Papanicolaou test because one is not able to perform colposcopy and a radical hysterectomy. In the same way, one should not withhold asking these simple questions because one is not a certified sex therapist.

We feel that the sexual function inquiry should always be included in the review of systems. This study proposes a practical and reliable method to accomplish this.

We conclude that a simple questionnaire can be used effectively by all physicians as a screening test for sexual dysfunctions relating to heterosexual coital activity. Further research is required to confirm this hypothesis with varied patient populations and under different conditions of doctor-patient relationship.

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The genital tract in female children with imperforate anus

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A retrospective study was undertaken to determine the type and frequency of genital tract anomalies that occur in female children with imperforate anus. The functional outcome and optimal management of the genital tract anomalies were also studied. Primary vaginal anomalies occurred in 22 of 72 patients assessed, and uterine anomalies occurred in 18 of 51 patients assessed. For 17 patients, who were 13 years of age or older, information was available on genital tract function. Of these 17 patients, five had severe vaginal scarring. A previous vaginoplasty or cloacal abnormality was a predictor of poor vaginal function. It is suggested that vaginoplasty, in cases in which it is possible, be delayed until puberty. (Am J Obstet Gynecol 1985;151:169-71.)

Key words: Genital tract anomalies, imperforate anus, vaginoplasty

The development of the cloaca in the female embryo is closely associated with the müllerian duct system. Thus abnormalities in the development of the urorectal system may have profound effects on the female reproductive tract.

Although much information is available as to the type, frequency, and treatment of urinary and rectal anomalies in imperforate anus, less is known of the genital anomalies that may occur. Even less is known about the treatment of these genital anomalies or the potential for future coital or reproductive functions.

We consequently set out to determine the types and frequency of genital tract anomalies that occurred in female children and to determine the functional outcome of their reproductive tract. We further hoped to draw some conclusions as to the optimum time for corrective surgery on the genital tract.

Table I. Distribution of anomalies of rectum and anus in 162 patients

Type of anomaly	*No. of patients*	%
Noncommunicating		
Anal stenosis	14	8.6
Imperforate anal membrane	3	1.9
Anal agenesis	11	6.8
Rectal agenesis	4	2.5
Rectal atresia	1	0.6
Unknown	1	0.6
Subtotal	34	21.0
Communicating		
Ectopic perineal anus	25	15.4
Covered anus	17	10.6
Rectovestibular fistula	37	22.8
Low rectovaginal fistula	13	8.0
High rectovaginal fistula	14	8.6
Cloaca	22	13.6
Subtotal	128	79.0
Total	162	100.0

Material and methods

A retrospective study of 162 female patients born after 1959 and treated for imperforate anus at The Hospital for Sick Children, Toronto, was undertaken to ascertain the type and frequency of genital tract anomalies.

Stephens'[1] classification of imperforate anus was used

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Table II. Morphology of the lower genital tract

Lower genital tract abnormalities	*No.*
Vaginal septum	13
Vaginal agenesis	5
Imperforate hymen	1
Distal stenosis	1
Absent lower third vagina	1
Hymenal band	1
Total	22

Table III. Morphology of the upper genital tract

Upper genital tract abnormality	*No.*
Bicornuate uterus	8
Uterus didelphys	8
Hypoplastic uterus	2
Total	18

in documenting the type of anal or rectal anomaly present. This classification was chosen because it divides these abnormalities into those that communicate (with vagina, vestibule, or perineum) and those that do not. This was thought to be significant with respect to vaginal function and outcome, since some girls with otherwise normal lower genital tracts may have rectovaginal fistulae.

The type of vaginal or uterine anomaly present was determined from the patient's chart by any examination or investigation that had been performed on the child or from operative notes or postmortem examinations.

The functional outcome of the reproductive tract was determined whenever possible in those girls who were 13 years of age or older. The outcome was classified as normal, mildly impaired (some scarring present but not necessitating future surgery), severly impaired (much scarring present which will require future surgery to ensure a functional outcome), and unknown.

Results

According to Stephens' classification, there were 34 patients with a noncommunicating anomaly (21%) and 128 patients with a communicating anomaly (79%). The actual type of anomaly is summarized in Table I.

The vagina was assessed in 72 of the 162 patients, with 22 having primary vaginal anomalies (i.e., excluding rectovaginal fistula) (Table II).

Documentation of the upper genital tract was available for 51 patients, with 18 of them having anomalies (Table III).

Sixty-six patients, 13 years of age or older, were studied in respect to genital tract function. Information was available for only 17 patients (Table IV).

Table IV. Genital tract function

Functional status	*No.*
Normal	9
Mildly impaired	3
Severely impaired	5
Unknown	49
Total	66

Data available in the patients' charts was insufficient to comment on coital or reproductive functions. However, over 85% of patients had normal or near-normal bowel, renal, and urinary function.

Comment

Although information was generally available on gastrointestinal and genitourinary abnormalities and functions, there was often little or no information available on these patients' genital tracts.

This is understandable since normal bowel and urinary continence was obviously more important than the future genital tract function in children. Nevertheless, the information that was available does give an indication of the genital tract abnormalities and functions that may be encountered in these girls.

Of the 162 patients, 72 had an assessment of the vagina, with 22 of these patients (32%) having primary vaginal abnormalities. A vaginal septum and vaginal agenesis were the most common findings. However, the total number of patients who had vaginal anomalies was much higher, since a communicating type of rectal anomaly with the vagina, vestibule, or perineum was present in 79% of the 162 patients. Although the communicating type of anomaly may be advantageous insofar as the bowel remains decompressed, it does mean that reconstructive surgery to the bowel also involves surgery to the lower genital tract with the risk of scarring.

Of the 162 patients, 51 had an assessment of the upper genital tract, with 18 (35%) showing abnormalities of the müllerian system. A bicornuate uterus and uterus didelphys were the most common abnormalities.

As a result of the advances in corrective surgery that have been made in recent years,[2-4] over 85% of the patients achieved normal or near normal bowel, renal or bladder function.

Over 90% of the 162 patients had surgery that involved the perineum, with all its ramifications on reproductive and coital function. Of the 66 patients 13 years of age or older who were assessed for vaginal function, there were 17 for whom information was available and five (29%) who had severe vaginal scarring. From these 17 cases it appeared that a previous vaginoplasty or cloacal abnormality was a predictor of poor vaginal function.

It is our impression that the initial attempt at vaginoplasty significantly contributes to scar tissue formation because the surgery is performed on unestrogenized tissues.

Although there were insufficient data to enable comment on coital and reproductive function, 29% of the patients for whom information was available did have severe vaginal scarring. Similarly, uterine abnormalities occurred in 35% of those for whom information was available. These uterine abnormalities have been shown to increase the rate of spontaneous abortion and premature labor and decrease the live-born rate.[5]

To ensure that the patients achieve normal coital and reproductive function, it is suggested that the genital tract be evaluated both at an early stage of management and again after the operation. Furthermore, vaginoplasty, if possible, is best left until the child has reached puberty, when the tissues are under the influence of estrogen and are more pliant and more amenable to surgical manipulation. It is hoped this would result in minimal scar tissue formation. The older child may also be able to cooperate during her postsurgical care, which may involve the use of a vaginal mold or dilators.

It is suggested that the gynecologist be involved at an early stage in the investigation and care of these infants with imperforate anus. This should ensure optimal bowel and urinary function while also preserving the anatomy and function of the reproductive tract.

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Comparison of the outcome of ovulation induction therapy in an in vitro fertilization program employing a low-dose and an individually adjusted high-dose schedule of human menopausal gonadotropins

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A low-dose "nonsuperovulation" scheme of ovulation induction (schedule 1: 15 treatment cycles in 13 women) was compared to a high-dose, individually adjusted "controlled-superovulation" scheme of human menopausal gonadotropin administration (schedule 2: 18 treatment cycles in 17 women, with four women also having been treated under schedule 1). In schedule 2 more human menopausal gonadotropin was employed, 17β-estradiol plasma levels were higher, and more ova were retrieved per treatment cycle ($p < 0.01$) with a higher rate of mature ova ($p = 0.001$). Also, under this schedule all treatment cycles resulted in successful retrieval of ova, whereas under schedule 1, four cycles (27%) were abandoned before laparoscopy because the follicles had ovulated ($p = 0.03$). Furthermore, the proportion of embryos cleaving to the two- to four-cell stage were higher under schedule 2 (38 of 53, or 72%) as compared to schedule 1 (13 of 25, or 52%), but this was not statistically significant ($p = 0.07$). Two pregnancies were conceived under schedule 2 giving a rate of 11% per treatment cycle. It was concluded that schedule 2, the individually adjusted controlled-superovulation scheme of human menopausal gonadotropin administration, was the superior technique of ovulation induction in an in vitro fertilization program. (AM J OBSTET GYNECOL 1985;151:172-5.)

Key words: Ovulation induction therapy, human menopausal gonadotropins, human chorionic gonadotropins, in vitro fertilization

Various techniques have been employed for the stimulation of multiple oocyte retrieval prior to in vitro fertilization.[1, 2] It is now also appreciated that the pregnancy rate is related to the number of embryos transferred to the uterus of the mother.[2-4] Human menopausal gonadotropin and human chorionic gonadotropin are potent, naturally occurring gonadotropins that have been employed for clinical induction of ovulation. This technique allows for superovulation[5] with consistent results in the recovery of oocytes by

Table I. Outcome of ovulation induction employing a low-dose nonsuperovulation and a high-dose, individually adjusted controlled-superovulation schedule of human menopausal gonadotropin administration

*Schedule**	*Human menopausal gonadotropin dose in ampuls*	*Plasma estradiol-17β (pg/ml)*	*Cycles in which ova were not obtained*		*Total ova*
			No.	*%*	
Schedule 1 (15 cycles in 13 women)	10.3 ± 0.4	343 ± 43	4/15†	27	25
Schedule 2 (18 cycles in 17 women)	19.8 ± 0.7‡	1288 ± 100‡	0/18§	0	53

*Four women were also treated in schedule 1. Total number of women treated in both schedules was 26.
†Abandoned before laparoscopy.
‡$p < 0.01$, Mann-Whitney test.
§$p = 0.03$, Fisher's exact test.
‖$p = 0.001$, Fisher's exact test.

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laparoscopy.[1,2] It also provides good control of follicular maturation with a low rate of luteinizing hormone surges during stimulation.[1,5] Simplified monitoring techniques and a greater flexibility in the timing of laparoscopy for oocyte retrieval may thus be possible. The latter has important practical advantages in the schedules of the operating room, the clinical staff, and the laboratory staff of a busy hospital. However, human menopausal gonadotropin is expensive; a low-dose schedule of administration would be desirable if it would achieve results equivalent to those of a high-dose scheme.

When the in vitro fertilization program was initiated at the University of British Columbia in July, 1982, the outcome of a low-dose "nonsuperovulation" schedule of human menopausal gonadotropin administration was compared to that of a high-dose, individually adjusted "controlled-superovulation scheme." The results, analyzed to November, 1983, a 17-month period, are presented in this paper.

Material and methods

Twenty-six women between the ages of 24 to 34 years with primary and secondary infertility due to inoperable tubal disease were treated over 33 cycles with human menopausal gonadotropin and human chorionic gonadotropin. The first 13 patients (31.4 ± 0.6 yr, mean ± SEM) were treated over 15 cycles according to schedule 1, the nonsuperovulation scheme. Pergonal, 1 to 2 ampuls daily, was given intramuscularly commencing between days 3 and 5 of the cycle until ultrasound studies showed the leading follicle(s) to be ≥1.5 cm in size, at which time 4000 IU of human chorionic gonadotropin was given intramuscularly. The human chorionic gonadotropin dosage given in this schedule was according to that employed by Marrs et al.[6] In this schedule no attempt was made to stimulate plasma estradiol-17β levels beyond the upper normal limit of 600 pg/ml. The remaining 13 patients and another four patients previously treated under schedule 1 (mean age, 30.7 ± 0.8 yr) were treated over 18 cycles under schedule 2, the controlled-superovulation scheme. Pergonal was administered intramuscularly from day 4 starting with 3 ampuls daily. The dose was reduced or maintained at this level depending on the plasma estradiol-17β and ultrasound findings. When plasma estradiol-17β concentrations reached approximately 1000 pg/ml and ultrasound studies showed the leading follicle(s) to be ≥1.5 cm, 5000 IU of human chorionic gonadotropin was administered intramuscularly. The human chorionic gonadotropin dosage employed in this schedule was according to that described by Lopata.[2] In this schedule the aim was to stimulate plasma estradiol-17β levels well above normal unstimulated levels of 600 pg/ml, i.e., controlled superovulation. Under both treatment schedules, laparoscopy and oocyte retrieval were performed 32 to 36 hours after human chorionic gonadotropin was given. Laparoscopy was performed under general anesthesia, employing the three-puncture technique with carbon dioxide as the gas phase.

Oocytes were cultured in modified Ham's F10 medium supplemented with 50 mg/L of penicillin G, 1.68 g/L (20 mmol/L) of sodium bicarbonate, 507 mg/L (5 mmol/L) of potassium bicarbonate, and 245 mg/L (1 mmol/L) of Ca-lactate. The water used was glass-distilled five times and processed through activated carbon ion exchange resin. The osmolarity was adjusted to a dilution of 280 mosm per kg of water. The final pH was from 7.3 to 7.4 after equilibration with a 5% carbon dioxide/5% oxygen/90% nitrogen gas mixture. The maternal serum used was heat-inactivated at 56° C for 30 minutes and filter-sterilized through a 0.22 μm membrane filter. The insemination media constituted 10% maternal serum. The growth media constituted 20% maternal serum.

Follicular fluids were scanned rapidly with a dissecting microscope. Once located, oocytes were graded for maturity on the basis of the presence or absence of the extracoronal cumular mass, morphologic characteristics of the corona cells, and the appearance of detached membrana granulosa cells. Preovulatory oocytes were routinely incubated for 6 to 8 hours prior to insemination, and immature oocytes were incubated approximately 24 hours prior to insemination.

Motile sperm from the husband's semen were ob-

	Mature ova		*Ova reaching 2- to 4-cell embryos*		*Fertilized and cleaving embryos transferred*		*Pregnancies*	
Ova per cycle	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1.8 ± 0.3	11/25	44	13/25	52	21/25	84	0/15	0
2.94 ± 0.4‡	46/53‖	87	38/53	72	43/53	81	2/18	11

tained by means of the overlay technique, with Ham's F10 medium. The sperm were washed twice with insemination medium. Approximately 10^5 sperm were used to fertilize each oocyte in 1 ml of insemination medium. About 16 to 20 hours after insemination, cumulus cells were removed (if necessary) by means of two 26-gauge needles. Fertilization was then demonstrated by the presence of pronuclei and two polar bodies, with the use of a dissecting microscope at 80 × magnification. The insemination medium was replaced with 1 ml of growth medium for further culture. Repeat examinations for cleavage were performed about 40 hours after insemination. Most of the embryos were transferred at the two- to four-cell stage.

Embryo transfers were performed with the patients in the lithotomy position. Open-ended Teflon catheters (William A. Cook Ltd., Surrey Hills, 3127 Victoria, Australia) were used. Embryos were drawn into the cannula, preloaded completely with maternal serum, and delivered into the uterine fundus with use of 25 to 50 μl of the medium. Blood samples were drawn every third to fourth day after embryo transfer for progesterone and human chorionic gonadotropin β-subunit assays.

Estradiol-17β was measured in plasma by radioimmunoassay employing a rapid technique. Assay kits, manufactured by Radio Isotopen Service/Isotopenproduktion Eidg. Instuit für Reaktorforschung 5303 Würenlingen, were purchased from Bionucleonics, Inc., Edmonton, Alberta, Canada. The mean within and between coefficients of variation were 6.3% and 9.3%, respectively. This is a double-antibody radioimmunoassay employing ^{125}I as the radioactive tracer. The first incubation period was 2 hours, and the second incubation period was 30 minutes. The assay was performed directly on aliquots of 50-100 μl of neat or diluted plasma. Blood samples were collected into 7 ml glass Vacutainer tubes (Becton Dickinson 6450) containing 0.07 ml of 15% ethylenediaminetetraacetic acid as anticoagulant. Human menopausal gonadotropin, Pergonal (75 IU FSH and 75 IU LH per ampul), was purchased from Serono Laboratories, Randolph, Massachusetts, while human chorionic gonadotropin was purchased from Ayerst Laboratories, Montreal, Canada. Data were analyzed by the Mann-Whitney test and Fisher's exact test.

Results

Results are shown in Table I. Compared to schedule 1, schedule 2 required more human menopausal gonadotropin; in the latter schedule, plasma estradiol-17β levels were higher, and more ova per treatment cycle and more mature ova were retrieved. In this schedule, all treatment cycles in which ovulation induction was initiated resulted in retrieval of ova, whereas in schedule 1, four cycles (27%) were abandoned before laparoscopy because the follicles had ovulated. Furthermore, the proportion of embryos cleaving to the two- to four-cell stage were higher under schedule 2 (38 of 53, or 72%) as compared to schedule 1 (13 of 25, or 52%) but this was not statistically significant ($p = 0.07$, Fisher's exact test). The rates of fertilization under both schedules were also similar. Two pregnancies were conceived under schedule 2 at a rate of 11% per treatment cycle.

Comment

The use of human menopausal gonadotropin in a high-dose, individually adjusted controlled-superovulation schedule, as compared to a lower-dose nonsuperovulation scheme, produced superior results. First, in schedule 2 (the controlled-superovulation scheme) ova were retrieved from all cycles in which ovulation induction therapy was initiated, whereas in schedule 1, 27% of treatment cycles were abandoned before laparoscopy because the follicles had ovulated during treatment. Second, schedule 2 produced significantly more ova, with a higher proportion of mature ova recovered per treatment cycle. Third, under schedule 2 more embryos reached the 2- to 4-cell stage after in vitro fertilization (72% vs. 52%, $p = 0.07$). It was of interest that in a series of carefully monitored human menopausal gonadotropin and human chorionic gonadotropin treatment cycles in anovulatory women (not in vitro fertilization–related indications) a similar controlled-superovulation schedule resulted in a 100% probability of ovulation occurring during treatment.[5] Despite the slightly higher human chorionic gonadotropin dosage employed, the improved results associated with schedule 2 were most likely the consequence of more advanced degrees of follicular stimulation achieved with this schedule.

Since the transfer of multiple embryos to the uterus of the mother influences the pregnancy rate,[2-4] a schedule of ovulation induction resulting in a high probability of retrieving ova, a high ova recovery rate, and a high proportion of cleaving embryos after in vitro fertilization would be advantageous. In this context a schedule of human menopausal gonadotropin administration based on the high-dose, individually adjusted controlled-superovulation mode appears to be superior to the lower-dose nonsuperovulation mode.

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Prostaglandins mediate postoperative pain in Falope ring sterilization

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To test the hypothesis that prostaglandins mediate the high incidence of postoperative pain in patients undergoing mechanical tubal occlusion, we studied the effect of premedication with a prostaglandin synthetase inhibitor in patients having Falope ring sterilization. Fifty-two women were randomly divided into two groups in which 19 (study group) received a preoperative indomethacin rectal suppository (100 mg) while 33 (control group) received nothing. Postoperative narcotic analgesics were administered as required, and for persistent pain an indomethacin suppository was given in the recovery room. Twenty-four percent of the control group required a postoperative indomethacin suppository because of persisting pain compared to none of the study group ($p < 0.02$). These results suggest that prostaglandins mediate, and premedication with a prostaglandin synthetase inhibitor may lessen, postoperative pain in patients having Falope ring sterilization. (AM J OBSTET GYNECOL 1985;151:175-7.)

Key words: Prostaglandins, Falope rings

Laparoscopic Falope ring tubal occlusion is a safe and effective method of sterilization but is associated with an incidence of severe postoperative pain ranging from <5% up to 33% in some studies.[1,2] Compared to other methods of tubal sterilization, the application of Falope rings results in a three to six times greater incidence of postoperative pain than electrocautery[2-4] and twice as frequent pain as tubal occlusion with Hulka clips.[3] Leggat and Barr[5] have suggested that the increased pain with mechanical occlusive procedures (compared to electrocoagulation) is secondary to ischemia and necrosis of the ligated portion of the tube without destruction of adjacent nerve fibers. However, a recent study demonstrated that postoperative pain associated with tubal occlusion was not improved by the application of lidocaine topically prior to ring application.[6]

Since prostaglandins are present in tubal tissue[7-9] and since tubal trauma or ischemia stimulate prostaglandin production,[10,11] we postulated that severe postoperative pain in patients undergoing Falope ring application may be mediated by prostaglandins. In the present study we examined the efficacy of the preoperative administration of a prostaglandin synthetase inhibitor in reducing the incidence of postoperative pain in patients undergoing Falope ring tubal occlusion.

Material and methods

Study subjects comprised 65 consecutive patients undergoing elective Falope ring sterilization over a 6-month period in one hospital. The patients were randomized by the senior operating room nurse on the basis of the last digit of their hospital number. Patients with an even number were placed in the study group while those with an odd number entered the control group. All patients were given a general anesthetic and endotracheal intubation with the usual preoperative

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Table I. The requirement for postoperative administration of an indomethacin suppository in the study group (group 1, premedication with indomethacin, 100 mg) and control group (group 2, no medication)*

	Postoperative indomethacin suppository			
	Required		*Not required*	
Group	*n*	*%*	*n*	*%*
1 (n = 19)	19	100	0	0
2 (n = 33)	25	76	8	24

*A postoperative indomethacin suppository was administered only if pain persisted after the standard narcotic administration in recovery room.

medications at the discretion of the attending anesthetist. Patients in the study group received a 100 mg indomethacin rectal suppository immediately after induction of anesthesia. Patients in the control group received nothing.

Although the laparoscopic procedures were carried out by different surgeons, the techniques used were quite similar. In the combined lithotomy-Trendelenburg position, the cervix was grasped with a tenaculum and a Cohn-Eder cannula positioned in the cervical os. Three liters of carbon dioxide gas was infused into the abdominal cavity under low pressure. The laparoscope was inserted through a subumbilical incision, and under direct vision a second trocar was inserted into the abdomen through a suprapubic incision to allow placement of the Falope ring applicator. After the pelvis was visualized and found to be normal, the fallopian tubes were identified, grasped in the midisthmic portion, and a Falope ring was applied so as to incorporate a complete loop of tube bilaterally. Following the procedure, the patient was taken to the recovery room where a standard protocol was followed for postoperative analgesia and patient care. The recovery room nurses were unaware that a study was being undertaken.

All patients received meperidine hydrochloride, 50 mg intramuscularly, as soon as possible after awakening. If the patient complained of severe pain 30 minutes later, a second intramuscular injection of meperidine hydrochloride, 50 mg, and promethazine hydrochloride, 25 mg, was given. Dimenhydrinate, 50 mg intravenously, was given once if necessary for nausea and vomiting. If the patient was still complaining of severe pain 30 minutes after the second injection of meperidine hydrochloride, a 100 mg indomethacin rectal suppository was administered. Diet was unrestricted while patients were in the recovery room, and they were discharged home as soon as they were able to leave.

Statistical analysis was by Cochran's modification of χ^2 and Student's t test for group data.

Results

At the end of the 6-month period, all of the charts were retrieved and the results tabulated. Of the 65 consecutive patients initially participating in the study, 13 were eliminated, 12 because of other operative procedures such as curettage performed along with the tubal occlusion and one because of failure to follow the protocol. The 52 patients ultimately remaining in the study, therefore, were women who underwent uncomplicated Falope ring tubal ligations as the only procedure. Of the 52 patients, 19 received a preoperative indomethacin suppository (the study group), while 33 (the control group) did not. The mean (±SEM) duration of operation for the study group (17.7 ± 3.5 minutes) and the control group (19.2 ± 2.9 min) was similar ($p < 0.05$).

As outlined in Table I, none of the 19 study patients required a second indomethacin suppository for pain control in the postoperative period. In contrast, eight of the 33 control patients (24%) had severe pain which was not controlled by 100 mg of meperidine hydrochloride and 25 mg of promethazine hydrochloride but was finally relieved by an indomethacin suppository. The difference between the two groups was statistically significant ($\chi^2 = 4.45$, $p < 0.02$).

Comment

The results of this study are in keeping with the previously reported high incidence of postoperative pain in patients undergoing Falope ring tubal occlusion.[2-4, 12] In the 33 control patients, 24% had postoperative pain that was not relieved by 100 mg of meperidine hydrochloride. Human fallopian tubes have been shown to produce prostaglandins[8, 9] which are not stored in tissues but are produced in response to trauma or other appropriate stimuli[10, 11, 13] It is possible that release of prostaglandins following the tubal trauma and ischemia induced by ring application acts locally to produce painful uterine contractions similar to those experienced by patients with dysmenorrhea.

Indomethacin is an effective inhibitor of the enzyme cyclooxygenase which is necessary for the production of prostaglandin $F_{2\alpha}$ and prostaglandin E (see reference 14 for review). Clinically, peak therapeutic blood levels of indomethacin are reached between 30 and 60 minutes following rectal administration[15, 16] and the half-life of the drug is estimated to be 8 to 10 hours.[17] Rectal suppositories were chosen for this study since the patients were allowed nothing by mouth prior to the surgical procedure and after operation the absorption of the indomethacin is not altered by vomiting. That the postoperative pain experienced by our patients was mediated by prostaglandins is suggested by the finding that indomethacin premedication prevented the necessity of extra analgesic in the study group.

The tubal occlusions in this study were all performed before peak concentrations of indomethacin would be expected (mean duration of operation was 17 ± 3.5 minutes versus 30 to 60 minutes for peak therapeutic levels). It is thus possible that some degree of prostaglandin release might have occurred prior to the onset of action of the indomethacin and that earlier pretreatment of these patients may markedly decrease the need for postoperative narcotics.

Although the number of patients in this study is small, the results strongly suggest that prostaglandins mediate, and premedication with a prostaglandin antagonist will relieve, much of the postoperative pain following Falope ring tubal occlusion. If indomethacin rectal suppositories are used we would recommend administration 1 to 2 hours prior to operation, that is, at the time of admission to the outpatient facility, in order to optimize the prostaglandin inhibition achieved at the time of tubal occlusion. Such premedication may significantly decrease the amount of narcotic analgesic required after operation and should facilitate earlier discharge of these patients.

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The effect of vaginal administration of various doses of prostaglandin E_2 gel on cervical ripening and induction of labor

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Eighty patients with a Bishop score of ≤ 4 were randomly administered vaginal triacetin gel containing placebo or prostaglandin E_2 in 1, 2, and 3 mg doses. Twelve to sixteen hours later, those not in labor underwent oxytocin induction. Increasing doses of prostaglandin were effective in ripening the cervix, and the higher doses were associated with significant success in inducing labor. (AM J OBSTET GYNECOL 1985;151:178-81.)

Key words: Prostaglandin E_2, cervical ripeness, labor induction

The degree of cervical ripeness correlates with the ease of induction and the duration of labor.[1] Induction of labor with an unripe cervix contributes to maternal and neonatal morbidity caused by prolonged labor and an increased need for operative delivery. Reports over the past several years have shown that prostaglandin E_2 in an intravaginal gel is effective in ripening the cervix without maternal, fetal, or neonatal complications.[2-5] This study compares the effects of the vaginal administration of prostaglandin E_2 gel in a single dose of 1, 2, or 3 mg to use of a placebo with respect to cervical ripening and the induction of labor.

Material and methods

Eighty singleton pregnancies requiring medical induction of labor were studied. After giving informed consent, patients were entered into a double-blind study and randomized into groups of 20. All patients had an initial Bishop[1] score of ≤ 4, were at ≥ 36 weeks' gestation, and were para 0 to 3. The indications for induction are listed in Table I. Exclusion criteria were as follows: (1) contraindication to vaginal delivery, (2) asthma or prior hypersensitivity to prostaglandins, (3) prior attempts at ripening or induction in this pregnancy, (4) malpresentation or multiple pregnancy, (5) intrauterine death, (6) polyhydramnios, (7) antepartum hemorrhage, (8) premature rupture of the membranes, and (9) uterine scar.

The gel was prepared in individual doses of 1.0 mg, 2.0 mg, and 3.0 mg of prostaglandin E_2 (Prostin E2) in a gel of triacetin and 10% colloidal silicone dioxide.* An identical-appearing placebo consisted of 5 ml of gel only. Each was premixed in a disposable syringe with a catheter for intravaginal insertion. Each syringe was sealed and stored at 4° C (stable for 15 months).† These randomly numbered identical syringes were dispensed in sequential order by the hospital pharmacy.

Each patient was assessed in the antenatal ward the evening before induction. A 30-minute observation period of external electronic fetal heart monitoring and uterine activity was required before gel insertion, and the patient was excluded if regular contractions were noted. If the Bishop score was ≤ 4, the gel was inserted into the posterior fornix through the disposable catheter. External electronic monitoring of the fetal heart and uterine activity was performed for 1 hour after insertion. If contractions were less frequent than two every 10 minutes and the fetal heart was normal, the patient was allowed to ambulate. The patient's temperature, blood pressure, and pulse were taken every hour for 3 hours and four times hourly thereafter. If labor occurred in the 12-to-16-hour observation period after gel insertion, the patient was transferred to the labor and delivery area. Amniotomy or augmentation of labor was left to the discretion of the attending staff.

Initiation of labor following the use of gel alone in this study was defined as regular uterine contractions (three in 10 minutes) without amniotomy, resulting in progressive cervical dilatation of ≥ 3 cm within the 12-

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Table I. Primary indications for induction

Indication	*Placebo*	*Prostaglandin E_2*		
		1 mg	*2 mg*	*3 mg*
Preeclampsia	5	11	12	8
Chronic hypertension	3	5	3	4
Prolonged pregnancy	9	1	1	3
Diabetes mellitus	3	1	2	–
Intrauterine growth retardation	–	2	2	2
Other	–	–	–	3
Total	20	20	20	20

Table II. Patient characteristics

	Placebo	*Prostaglandin E_2*		
		1 mg	*2 mg*	*3 mg*
No. of patients	20	20	20	20
Nulliparas	11	15	15	8
Multiparas	9	5	5	12
Age (mean yr)	26.8	27.2	24.7	27.3
Gestation (mean wk)	40.0	39.0	39.0	38.9
Initial Bishop score (mean)	2.4	2.7	3.0	2.6

Table III. Change in Bishop score

	Placebo	*Prostaglandin E_2*		
		1 mg	*2 mg*	*3 mg*
Mean initial Bishop score*	2.5	2.7	2.9	2.3
Mean Bishop score after receiving gel*	3.7	5.4	5.5	6.1
Mean change in Bishop score†	1.4	2.7	2.6	3.8
Number of patients*	20	18	15	9

*This table includes only those patients who were not delivered by the end of the observation period and who therefore had a second Bishop score.

†$p < 0.01$ by analysis of variance.

Table IV. Effect on induction and labor

	Placebo	*Prostaglandin E_2*		
		1 mg	*2 mg*	*3 mg*
Labor after gel alone	0/20	1/20	5/20	10/20
Gel induction–to–delivery interval (mean hr)	—	4.9	12.8	9.0
Successful oxytocin induction	14/19	11/18	9/12	5/6
Oxytocin induction–to–delivery interval (mean hr)	9.4	10.2	8.0	7.2
Total successes—gel and oxytocin induction	14	12	14	15
Delivery by cesarean section	3	7	5	4

to 16-hour observation period. Uterine activity was considered hypertonic if contraction frequency was more than five in 10 minutes or if an individual contraction exceeded 2 minutes' duration. If hypertonia occurred, the gel was removed with gauze swabs and saline irrigation.

If the patient was not in labor after 12 to 16 hours, then the same observer reassessed the Bishop score. Intravenous oxytocin induction of labor was then started, followed by amniotomy when cervical dilatation reached 3 to 4 cm. Induction of labor was considered successful when cervical dilatation reached 5 cm. If the patient was not in labor after 8 hours of oxytocin infusion or required cesarean section prior to 5 cm dilatation, the induction was considered a failure.

Fetal distress was defined as repeated late decelerations, persistent profound variable decelerations (<70 beats per minute for 60 seconds or longer), and fetal bradycardia or tachycardia combined with loss of baseline variability. One- and five-minute Apgar scores and the neonatal course to discharge from hospital were documented.

Results

The patient characteristics are listed in Table II; there were no differences between the groups with respect to mean maternal age, gestational age, or initial Bishop score. Of the 80 patients who received the gel, four were excluded during the observation period. One patient in each of the groups receiving Prostin E2 developed fetal distress and required cesarean section. One patient developed accelerated hypertension, and early induction with oxytocin removed her from the study. After exclusion of these cases and those who came into labor and delivered during the observation period, a second Bishop score assessment was performed on 62 patients. As can be seen in Table III, the prostaglandin E_2 gel in any dose was statistically superior to the placebo in improving the Bishop score ($p < 0.01$). The Prostin gel alone effectively induced labor in 16 patients, and this was dose related (Table IV). In spite of the cervical ripening, there was no apparent increase in success of oxytocin induction except in the 3 mg group where the numbers were too small to show statistical significance. From the results in Table IV, it can be seen that there was no significant difference between the placebo and Prostin groups in the interval from oxytocin induction to delivery. The dose of oxytocin required and the need for delivery by cesarean section did not vary appreciably between groups.

Complications are listed in Table V. The gel was removed in nine cases: seven had uterine hypertonia, one

Table V. Complications

	Placebo	Prostaglandin E_2		
		1 mg	*2 mg*	*3 mg*
Hypertonia	—	1	2	4
Fetal distress	—	1	1	1
Vulvar edema	—	—	1	—
Gastrointestinal side effects	—	1	6	1
Gel removal	—	2	3	4

had fetal distress associated with normal uterine activity, and one developed severe vulvar edema. More uterine hyperstimulation occurred with higher doses of prostaglandin E_2. The onset of hypertonia ranged from 4 to 8 hours except for one multiparous patient in whom it occurred within 5 minutes of 3 mg gel insertion. Hypertonia occurred in 18% of all multiparous patients and 8% of nulliparous patients receiving prostaglandin E_2.

Fetal distress was associated with two cases of hypertonia and one case with normal uterine activity. Cesarean sections were performed, and the 1- and 5-minute Apgar scores were >6 in each case. In the full study group of 80 there were three infants with 5-minute Apgar scores <6: one in each of the groups receiving placebo, 2 mg of gel, and 3 mg of gel. All had uneventful first stages of labor but were delivered by midforceps. Their subsequent neonatal courses were uneventful.

Mild nausea and vomiting occurred in six patients in the group receiving 2 mg of gel and in one patient in the group receiving 1 mg of gel. Diarrhea occurred in one patient in the group receiving 3 mg of gel. The gel was not removed for gastrointestinal complications in any of the patients. One patient in the group receiving 2 mg of gel complained of intense vulvovaginal pain within 1 hour of gel insertion. The residual gel was removed, but over the next 12 hours she developed severe vulvar edema, which decreased in the following 72 hours.

Comment

Prostin E2 gel applied intravaginally is effective in changing the preinduction Bishop scores at all doses, 1 to 3 mg, compared to placebo. The 3 mg dose of prostaglandin E_2 gel resulted in the onset of labor in 50% of patients.

Increasing doses of prostaglandin E_2 are associated with uterine hypertonia and the incidence is higher than in previous reports with gel. The gel was easily removed as described, and in five of seven cases normal uterine activity resumed. In all cases neonatal outcome was normal.

The optimum dose of intravaginal or intracervical prostaglandin E_2 gel for cervical ripening has not yet been defined. Only four cases of gel-induced hypertonia are reported in the literature.[3, 4, 6, 7] MacKenzie and Embrey[3] reported that a multigravid patient received 5 mg of prostaglandin E_2 in Tylose vaginally and developed hypertonia associated with nausea and vomiting. Based on eight patients in their study who had rapid and uncomfortable labors but no evidence of hypertonia, they administer only 2.5 mg of prostaglandin E_2 to multigravid women. The present study confirms that caution is warranted in administering large doses and that close surveillance is necessary.

The gel itself affects the rate of absorption. Human studies of prostaglandin E_2 absorption in Tylose gel demonstrate an extremely rapid rise in the 15-keto-prostaglandin E_2 metabolites in serum within the first hour after insertion. In some individuals, there is a second unexplained peak about 4 hours later.[8] Variation in absorption may account for more rapid onset of action in certain patients. Studies in monkeys indicate similar prostaglandin E_2 absorption from the vagina with Tylose and triacetin gels. The relatively high incidence of uterine hypertonia and gastrointestinal side effects in the present study suggests the possibility of increased absorption in humans using triacetin gel.

Case reports of vaginal irritation are rare and probably are related to idiosyncratic contact dermatitis. A few cases were reported of mild burning sensation of the vagina during the first hour after Tylose gel insertion.[3] One patient had marked vulvar erythema lasting 48 hours after extra-amniotic insertion of 3 mg of prostaglandin E_2 in 6% Tylose.[9]

Only two cases of nausea and vomiting have been reported with vaginal or intracervical gel, and in both instances 5 mg of prostaglandin E_2 was used.[3, 8] Uterine hypertonia was associated with both cases. The gastrointestinal side effects in the present study were not severe, and in only one instance was there associated hypertonia.

As a method of assisting induction with an unripe cervix, prostaglandin E_2 in triacetin gel shows promise. There were clinically significant changes in the Bishop score (a change of ≥4) in only 10% of placebo recipients versus 78% of recipients of 3 mg of gel. There were insufficient numbers to demonstrate statistical significance in a comparison of oxytocin induction successes, since 27% of the prostaglandin E_2 group had delivered with gel alone. There was a 33% failed-induction rate in the nulliparous patients who received prostaglandin E_2 and a 10% rate in the multiparous patients. This did not differ significantly from the control group. Similarly, the cesarean section rate was not appreciably different between groups and reflected the high-risk nature of this population and the difficulty of successful induction.

This study shows that the intravaginal application of prostaglandin E_2 in triacetin gel is effective in ripening the unfavorable cervix and that the 3 mg dose alone will induce labor in a significant number of patients. Caution is recommended because of the risks of hypertonia in patients with marginal uteroplacental perfusion.

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Fetal and neonatal effects of indomethacin used as a tocolytic agent

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β-Mimetic agents are currently recommended for tocolysis. Serious adverse reactions including maternal death may complicate their use. Prostaglandin synthetase inhibitors are effective tocolytic agents. Reports of potential adverse effects have limited clinical use in North America. This study reports the perinatal outcome in 167 infants exposed to indomethacin used for tocolysis in gestations of less than 35 weeks. The rate of preterm delivery was 41.3% (69 of 167). No cases of premature closure of the ductus arteriosus or persistent fetal circulation were observed. The overall perinatal mortality was 17 per 1000 (1.7%). The results support the view of other uncontrolled reports that a short course of indomethacin used for tocolysis in gestations of less than 34 weeks is without deleterious effects on fetus or neonate. Its ease of administration and maternal safety offer advantages over β-mimetic agents, and prospective comparisons of both should be undertaken. (AM J OBSTET GYNECOL 1985;151:181-4.)

Key words: β-Mimetics, indomethacin, tocolytic agents, prostaglandin synthetase inhibitors, preterm labor

Current epidemiologic studies identify only a minority of patients at risk for preterm labor. Therapeutic strategy for reducing preterm births thus emphasizes tocolysis over prophylaxis. β-Mimetic agents are proven uterine relaxants[1] and have been used for over two decades. Numerous reports confirm their ability to prolong pregnancy,[2, 3] but few studies demonstrate a decrease in the incidence of preterm births.[1] Improved perinatal outcome should be the measure of efficacy, but only one study contains significant information on outcome.[4] The uncertainty of whether tocolysis improves perinatal outcome makes the issue of fetal and maternal drug toxicity more important. β-Mimetic agents are associated with serious complications, including maternal death.[5] Isoxsuprine, the least β_2 selective of available agents, is associated with a high rate of side effects and low tocolytic efficacy.

The unfavorable risk/benefit ratio of isoxsuprine prompted the use of indomethacin, a prostaglandin synthetase inhibitor, as an alternative in our hospital. Case reports of potentially adverse fetal effects[6-8] have limited the clinical use in North America despite contradictory evidence from several uncontrolled studies that indomethacin is an effective tocolytic without

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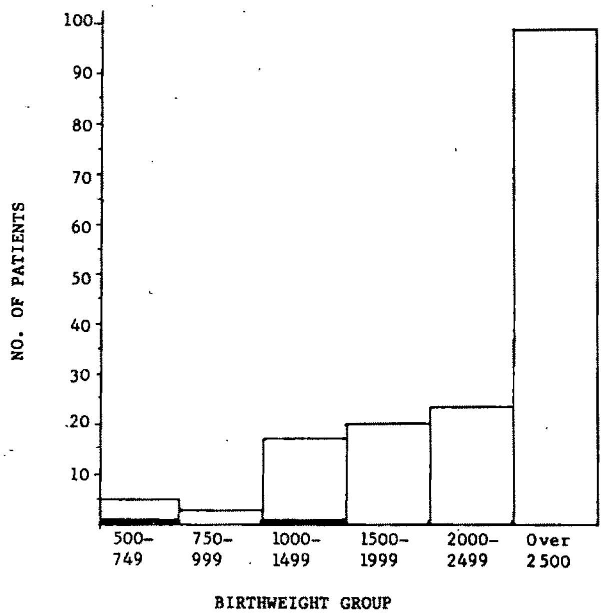

Fig. 1. Birth weight distribution and perinatal outcome. *Shaded area* represents number of neonatal deaths (total, 2).

harmful effects.[9-11] This report describes our experience and the observed perinatal effects in 167 patients treated with indomethacin from January, 1980, to January, 1983. It presents evidence that the drug is unassociated with any significant adverse fetal effects and discusses its potential advantages over currently approved β-mimetic agents.

Material and methods

One hundred sixty-seven patients received indomethacin for tocolysis in a 3-year period. All were of gestational age of 20 to 35 weeks. Treatment was started for regular uterine contractions >30 seconds in duration at least twice every 15 minutes or for progressive cervical effacement of dilatation. Cervical dilatation >4 cm excluded patients from treatment. Fifteen patients in whom β-mimetics were contraindicated received therapy with indomethacin. Forty-five (27%) of 167 patients had ruptured membranes. Thirty-seven patients (22.1%) received intravenous isoxsuprine and indomethacin concurrently.

Indomethacin, 100 mg, was administered rectally and repeated in 1-2 hours if regular uterine contractions persisted. At the same time a regimen of 25 mg orally every 6 hours for 24 to 48 hours was started. Therapy was usually limited to 24-48 hours, but eight patients received courses of oral therapy for periods ranging from 3 to 9 days. The average quantity of indomethacin received was 225 mg. The mean duration of therapy was 38.5 hours. One patient with ruptured membranes and preterm labor at 23 weeks' gestation was treated with 25 mg orally four times daily until delivery at 30 weeks.

All preterm infants were admitted to an intensive care nursery and observed for any evidence of persistent fetal circulation or prenatal closure of the ductus arteriosus. This included clinical assessment, chest x-ray examination, and blood gas determination. Renal function, coagulation parameters, and bilirubin levels, although assessed on an ongoing basis, were also analyzed retrospectively from chart review. A chart review was used to study outcome in infants whose mothers were treated for preterm labor but delivered at term. These neonates were admitted to a regular nursery and evaluated by an attending pediatrician. Maternal side effects, including gastrointestinal and hematologic changes and the incidence of postpartum hemorrhage, were also analyzed from the chart review.

Perinatal mortality consisted of fetal deaths plus neonatal deaths within 28 days.

Results

Delivery was postponed for >72 hours in 132 of 167 patients (79%). In the group of 35 patients who delivered within 72 hours the mean interval between last treatment and delivery was 18.6 hours. Delivery was postponed for >1 week in 112 of 167 cases (67.2%). Of these 112 patients, 104 delivered at term and the mean interval between last treatment and delivery was 6.7 weeks. Of the 55 patients who delivered within 1 week, 41 (74.5%) had ruptured membranes. Of the 41 patients with ruptured membranes, 36 (80%) delivered in 72 hours and five more within 1 week. Success, defined as delaying delivery for >1 week, was 64.6% with intact membranes versus 8% when amniorrhexis occurred. The mean gestational age at entry into the study was 30.8 weeks compared with a mean gestational age at delivery of 35.6 weeks.

Fig. 1 shows birth weight at delivery related to perinatal outcome. There were two neonatal deaths, giving an overall perinatal mortality of 17 per 1000 (1.7%). Neither was attributed to any possible adverse effects of indomethacin, and both were in patients who also received courses of isoxsuprine. Both patients had associated amniorrhexis and one mother was the patient who received indomethacin for >6 weeks. Table I summarizes these two neonatal deaths.

No cases of persistent fetal circulation, prenatal closure of the ductus, or neonatal platelet dysfunction were observed. Neonatal complications occurred in 38% of preterm deliveries, including cases of respiratory distress syndrome, hypoglycemia, hyperbilirubinemia, and necrotizing enterocolitis and one case of infantile diabetes mellitus, and are shown in Table II. There was no correlation between neonatal morbidity

Table I. Summary of perinatal deaths

Birth weight (gm)	*Gestational age (wk)*	*Therapy*	*Duration*	*Cause of death*
570	25+	Indomethacin; Vasodilan*	12 hr	Respiratory distress syndrome; immaturity
1210	30+	Indomethacin; Vasodilan†	6 wk	Sepsis; pulmonary hypoplasia

*Vasodilan (isoxsuprine) given intravenously for 6 hr.

†Vasodilan given intravenously at 23+ and 26 weeks for 6-12 hr.

and the length of the interval between last treatment and delivery. No cases of neonatal sepsis were recorded in surviving infants.

Maternal side effects were limited to two patients with upper gastrointestinal irritation after oral indomethacin and four who experienced diarrhea after suppository administration. Three patients (1.7%) were recorded as having postpartum hemorrhage. All delivered >4 weeks after receiving indomethacin, which would eliminate the drug as a causative factor.

Table II. Neonatal complications*

Complication	*No. of patients*
Respiratory distress syndrome	22
Hypoglycemia	14
Necrotizing enterocolitis	5
Infantile diabetes mellitus	1
Hyperbilirubinemia†	16
Thrombocytopenia	0

*One or more of the complications listed occurred in 27 of 69 (38%) preterm infants.

†Defined as a bilirubin level of >170 μmol/L. Three infants required exchange transfusion.

Comment

Indomethacin has been reported to be of the same level of efficacy as β-mimetics.[9-11] The only study comparing indomethacin with ritodrine or placebo favored indomethacin significantly.[9] Our study, being retrospective and uncontrolled, cannot address the question of tocolytic potential or perinatal outcome. Its sole purpose is to evaluate the potential adverse fetal effects that continue to limit clinical use in North America. Persistent fetal circulation and prenatal closure of the ductus arteriosus are the two major concerns although other side effects could occur. Neither complication was observed in our study.

Persistent fetal circulation is a syndrome of cyanosis and cardiopulmonary disturbance which may be primary or secondary to perinatal asphyxia or sepsis, meconium aspiration, diaphragmatic hernia, pulmonary hypoplasia and, allegedly, prostaglandin synthetase inhibitors. Most cases of persistent fetal circulation associated with prostaglandin synthetase inhibitors are reported with chronic administration beyond 34 weeks' gestation or with concomitant risk factors for persistent fetal circulation such as sepsis or perinatal asphyxia. The evidence incriminating indomethacin is imprecise. Manchester et al.[6] reported a possible association between maternal indomethacin therapy for 4 days and persistent fetal circulation in two neonates. One infant had sepsis and both suffered birth asphyxia, which makes it difficult to definitely attribute these cases to indomethacin exposure. Similar vague case reports describe "difficult oxygenation at birth"[7] or symptoms consistent with disturbed cardiopulmonary adaptation[12] with no confirmatory studies such as echocardiography. All were neonates prenatally exposed beyond 34 weeks' gestation with birth weights >2200 gm.

Prenatal closure of the ductus is a rare condition that was observed from 800 perinatal autopsies. One case associated with maternal salicylate therapy for 2 weeks until delivery at 37 weeks' gestation has been reported.[13] Case reports of adverse effects following salicylate therapy cannot be extrapolated to indomethacin. Salicylates inhibit prostaglandin synthesis by irreversible inactivation of the enzyme cyclooxygenase whereas indomethacin does so reversibly. In animals the response of the ductus in utero to aspirin differs from response to indomethacin. Whereas aspirin causes constriction of the ductus in rats at any gestational age, the response to indomethacin varies with gestational age.[14] Animal data suggesting that the ductus arteriosus in the immature fetus is insensitive to effects of indomethacin[15] are also supported by observations in human neonates.[16] A recent study by Klein et al.[17] indicates that indomethacin does not cross the rat placenta significantly until near term. This provides a better explanation than changing sensitivity of the ductus for the absence of fetal effects in immature gestation. It was assumed that indomethacin freely crossed the placenta from animal studies in which the drug was administered in late gestation and its effect on ductus closure noted.[15] Few studies measure placental transfer directly as in Klein's study, and all were in term pregnancies. Studies in humans of placental transfer of indomethacin are rare and also pertain only to term gestation.

Several moderate size studies[9, 10] supported by available animal data indicate the drug to be safe, contradicting isolated case reports. Niebyl[18] concluded from reviewing the literature that a 24-hour course of indomethacin in gestations of <34 weeks minimizes any risks and could justify clinical use. Betamethasone is used to enhance pulmonary maturity and could have possible effects on ductus closure of persistent fetal circulation. To our knowledge there have been no reports of these adverse effects associated with betamethasone administration in pregnancy. Steroids act at a higher rate-limiting step to decrease prostaglandin synthesis by inhibiting phospholipase A_2. It is difficult to see why a 24-hour course of indomethacin (which may not cross the placenta in immature gestation) should be potentially more harmful than a 24-hour course of betamethasone.

Neonatal bleeding or renal impairment could potentially result from tocolytic therapy with indomethacin. However, effects of platelet function are more likely with aspirin and are probably rare with indomethacin because of its reversible action. Bilirubin toxicity from drug-induced displacement of bilirubin from binding sites on albumin could occur, but short-term use should again minimize this risk. The incidence of hyperbilirubinemia in this study did not seem excessive.

The ease of administration makes indomethacin an attractive alternative to β-mimetic therapy. Intravenous infusion of a β-agonist requires continuous medical supervision with serial and multiple laboratory tests in an intensive care setting. Our study confirms that maternal safety with indomethacin therapy is an advantage over β-mimetic administration associated with more numerous, more frequent, and occasionally fatal side effects.

Our results suggest that short term indomethacin used for tocolysis does not adversely affect neonatal outcome. The two neonatal deaths in our series were not attributable to indomethacin although it is possible that the drug masked chorioamnionitis in the patient treated for 6 weeks.

Indomethacin may be preferable when β-mimetics, but not tocolysis, are contraindicated. It should not be used in patients with bleeding disorders, renal disease, or peptic ulceration. Its antipyretic effect may mask chorioamnionitis, necessitating caution in its use if amniorrhexis is present. Limiting its use to early gestation when tocolysis is most appropriate minimizes any potential hazards. Efficacy and risk/benefit considerations seem to justify a prospective randomized comparison of indomethacin to currently approved β-mimetic agents. This study could also assess the placental transfer of indomethacin in human preterm gestation and ultimately resolve the issue of fetal toxicity.

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The association of sexually transmitted diseases with cervical intraepithelial neoplasia: A case-control study

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Thirty-three women with histologically confirmed cervical intraepithelial neoplasia (grades I to III, with one case of microinvasive carcinoma) and 54 women without evidence of the disease were prospectively studied to determine the relationship of genital infection to cervical neoplasia. Demographic and sexual data for patients and control subjects were collected, with standardized clinical and colposcopic evaluation by means of predefined diagnostic categories. Cultures from the cervix were examined for herpes simplex virus, cytomegalovirus, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*. Human papilloma virus infection was identified by characteristic changes of koilocytosis in cytologic or histopathologic specimen. Cultures from the vagina were evaluated for *Gardnerella vaginalis, Trichomonas vaginalis, Ureaplasma urealyticum, Mycoplasma hominis, Candida albicans*, and other yeasts. Separate Gram strains were prepared from endocervical secretions and from vaginal secretions. More lifetime sexual partners, larger area of transformation zone, evidence of human papilloma virus infection, and altered vaginal flora were observed in women with cervical intraepithelial neoplasia. The association of human papilloma virus infection and altered vaginal flora with cervical intraepithelial neoplasia was independent of sexual experience. (AM J OBSTET GYNECOL 1985;151:185-90.)

Key words: Sexually transmitted diseases, cervical intraepithelial neoplasia, human papilloma virus infection, altered vaginal flora

Cervical intraepithelial neoplasia behaves as a sexually transmitted disease, for which one or several microbial etiologies seem likely.[1] Exposure to putative microbiologic agents during coitus at an early age or through multiple sex partners appears to be the key biologic event.

The pathogenesis of cervical neoplasia is best explained with a two-hit model: metaplastic epithelium at the squamocolumnar junction of the cervix acquires the capacity for neoplasia after an "initiating" event. The initiator or promoter or both are hypothesized to be transmitted through coitus.

At least eight microbial agents transmissible through coitus have been incriminated in cervical neoplasia, i.e., *Chlamydia trachomatis, Neisseria gonorrhoeae, Gardnerella vaginalis, Mycoplasma hominis, Trichomonas vaginalis*, herpes simplex virus, cytomegalovirus, and human papilloma virus. The roles of herpes simplex virus type 2[2] and of human papilloma virus[3] in cervical neoplasia appear strongest; multiple and possible interacting microbial etiologies also appear likely.

Most etiologic studies of cervical neoplasia to date have examined the role of a single or, at best, a few microbial agents. Since sexually transmitted diseases frequently coexist,[4] the association of one agent with the diseases does not constitute strong causal evidence unless multiple agents are simultaneously studied.

We conducted a case-control study of women with and without cervical intraepithelial neoplasia for the prevalence of infection with a variety of sexually transmitted disease pathogens and for the prevalence of bacterial vaginosis and mucopurulent cervicitis to determine which of these pathogens or clinical syndromes is associated with cervical intraepithelial neoplasia.

Material and methods

Patient population. Forty-eight women were referred to the Colposcopy Clinic, Women's Hospital, Health Sciences Centre, Winnipeg, Manitoba, for colposcopic evaluation because two recent Papanicolaou smears showed changes consistent with grade I or worse. Of these 48 women 33 were confirmed to have a cervical intraepithelial neoplasia lesion at enrollment, and 15 were found to be free of disease. Thus 33 women with documented cervical intraepithelial neoplasia lesions are included as case subjects in this study.

Sixty-one women attending the Family Planning Clinic adjacent to the Colposcopy Clinic were evaluated

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Table I. Demographic and behavioral data

	Controls (n = 54)	*Cases with cervical neoplasia (n = 33)*	*p*
Age (yr) (mean ± SD)	21.9 ± 4.0	27.0 ± 12.1	0.035
No. of pregnancies (mean ± SD)	1.2 ± 1.4	2.8 ± 2.5	0.001
Income <$10,000/year (%)	37 (68)	26 (79)	0.3
No. of single women (%)	38 (76)	20 (61)	0.05
No. of smokers (%)	29 (55)	22 (70)	0.17
Lifetime number of sex partners (mean ± SD)	6.6 ± 6.7	15.6 ± 23.7	0.04
Age (yr) of first intercourse (mean ± SD)	16.2 ± 2.1	15.4 ± 3.5	0.23

for a comparison group. This population was felt to be a reasonable control group, since they represented sexually active individuals of approximately the same socioeconomic group. Women were selected in a nonrandom but nonsystematic manner. Informed consent was obtained from these participants after review of the protocol by the Institutional Review Committee of the University of Manitoba. Seven (11%) of these 61 women had cervical intraepithelial neoplasia lesions at the time of enrollment (six had grade I lesions and one had a grade II lesion). The remaining 54 women were free of disease and served as control subjects.

Clinical evaluation. A standardized history and physical examination were performed on each study participant. Historic information elicited included demographic data, obstetric history, sexual history, age of first intercourse, lifetime numbers of sexual partners, and years of sexual activity.

The vulva was inspected for evidence of genital ulceration and condyloma acuminata. Following insertion of the lubricant-free vaginal speculum, the appearance of vagina secretions was noted. Vaginal fluid pH was measured by means of pH strips (color pHast, pH 4.0 to 7.0, MCB Reagents, Gibbstown, New Jersey), and a sample of secretions was collected for the amine odor test and for wet-mount microscopy. If a strong "fishy" odor was volatilized on mixing of vaginal fluid with 10% potassium hydroxide, the amine odor test was said to be positive. Under wet-mount microscopy motile trichomonads, clue cells, and pseudohyphae of yeast were sought. Bacterial vaginosis (nonspecific vaginitis) was said to be present if three or more of the following four criteria were present: thin adherent homogenous vaginal discharge, pH ≥4.7, positive amine odor test, or clue cells.

The cervix was exposed and a Papanicolaou smear obtained. A cotton-tipped swab was inserted into the endocervical canal, and the presence of yellow secretions on the swab tip defined mucopus. A Gram stain of endocervical secretions was then prepared. Colposcopy was performed with a Zeiss colposcope, and findings were recorded according to the nomenclature adopted at the Second World Congress of Colposcopy in Graz, Austria, in 1975.[5] The cervix was examined with a green filter to determine the presence or absence of atypical vessels. Then 3% acetic acid solution was applied to the cervix to remove excess mucus and accentuate differences between normal and abnormal colposcopic patterns. During colposcopy special emphasis was given to the topography and color of the epithelial surfaces and the underlying vascular architecture. Colposcopically directed biopsies were taken when indicated.

Microbiologic methods. Cultures for the following organisms were obtained: *Chlamydia trachomatis, Neisseria gonorrhoeae, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum, Trichomonas vaginalis, Candida albicans,* herpes simplex virus, and cytomegalovirus. Type III Calgiswabs were used to collect serial specimens from the endocervix and vaginal secretions.

C. trachomatis was isolated in Hela 229 tissue culture. From 48 to 72 hours after infection, inclusions were identified by immunofluorescence by the use of a monoclonal antibody to a species antigen of *C. trachomatis. N. gonorrhoeae* was identified on a Thayer-Martin/chocolate split plate. After 48 hours' incubation at 36° C in a carbon dioxide candle extinction jar, *N. gonorrhoeae* was identified by its Gram stain morphology and by a positive oxidase reaction and was confirmed by sugar utilization tests. A swab of vaginal secretions was used for isolation of *G. vaginalis* on human blood bilayer Columbia agar plate containing amphotericin (2 mg/ml), colistin, and nalidixic acid. Plates were incubated in 5% carbon dioxide at 37° C and read at 48 and 72 hours. β-Hemolytic small white colonies containing pleomorphic gram-variable rods that were catalase negative and fermented starch were identified as *G. vaginalis. U. urealyticum* and *M. hominis* were identified from swab cultures of vaginal secretions. Swabs were used to inoculate A-7 and M agar and T and M-10 broth. Plates were incubated in a carbon dioxide jar at 37° C for up to 5 days and broths were incubated anaerobically at 37° C. Plates were first examined at 48 hours for typical colonial morphology. *M. hominis* was recognized by its "fried egg" appearance. *U. urealyticum* was identified by its small colony size and brown color on A-7 medium

Table II. Bacterial vaginosis

	Controls (n = 52)	*Cases with cervical neoplasia (n = 31)*	*p*
Vaginal pH (mean ± SD)	4.66 ± 0.51	4.81 ± 0.41	0.15
Bacterial vaginosis smear (%)	15 (29)	14 (45)	0.1
Lactobacilli predominant (%)	26 (50)	10 (33)	0.09
Vaginal flora			
Grade I	32	10	
Grade II	18	14	
Grade III	1	6	0.01

Table III. Colposcopic findings

	Controls	*Cases with cervical neoplasia*	*p*
Columnar epithelium (mean ± SD)	15.8 ± 8.5 (n = 50)	10.0 ± 4.6 (n = 29)	0.001
Transformation zone (mean ± SD)	24.9 ± 8.3 (n = 50)	30.0 ± 6.6 (n = 33)	0.0003
Atypical transformation zone	n = 50	n = 33	
White epithelium and/or keratosis (%)	6 (12)	22 (67)	0.0001
Mosaic and/or punctation (%)	1 (2)	10 (30)	0.0001
Atypical vessels (%)	0 (0)	1 (3)	
Any atypical transformation zone (%)	7 (14)	33 (100)	0.0001

Table IV. Microbiology of cervix

	Controls		*Cases with cervical neoplasia*		
	No.	*%*	*No.*	*%*	*p*
Human papilloma virus	2/54	4	15/33	45	0.0001
Chlamydia trachomatis	7/54	13	5/33	15	0.95
Neisseria gonorrhoeae	0/54	0	1/32	3	0.19
Herpes simplex virus	1/53	2	1/32	3.1	0.71
Cytomegalovirus	2/52	3.8	2/30	6.7	0.57

(indicating ammonia production from urea utilization). Swab cultures of vaginal secretions were used to inoculate Sabouraud's media for isolation of *C. albicans*. Identification of all yeast was based on API strip reactions. Swabs of vaginal secretions were used to inoculate modified Diamond's media containing horse serum, yeast extract, maltose, cysteine, ascorbic acid, and 1000 units/ml of penicillin G, 500 mg/ml of streptomycin, and 2 mg/ml of amphotericin. Cultures were incubated aerobically at 37° C and evaluated microscopically at 48 to 72 hours and 6 to 7 days for motile trichomonads. Swab cultures from the cervix were stored in viral transport media and used to inoculate human foreskin fibroblasts for isolation of herpes simplex virus and cytomegalovirus. These viruses were identified by their typical cytopathogenic effect.

Gram stains of endocervical secretions and of vaginal secretions were separately obtained. Endocervical secretion was examined for the number of polymorphonuclear leukocytes per oil immersion field (× 1000) and categorized as ≤10 or >10 polymorphonuclear leukocytes per × 1000 as a mean of five fields. Vaginal secretions were examined for the predominant flora and classified as follows: grade I if only gram-positive rods characteristic of lactobacilli were seen; grade II if gram-positive or gram-variable rods, gram-negative rods, and gram-positive cocci were seen; grade III if only gram-negative rods or cocci were seen. A smear compatible with bacterial vaginosis (nonspecific vaginitis) was noted if bacterial morphotypes suggestive of *G. vaginalis* (short gram-negative or variable rods) together with gram-negative rods and/or gram-positive cocci were seen.[6]

Cytopathologic studies

Cytology. Papanicolaou smears were obtained by a combined method of swabbing of the endocervical canal with a cotton swab and scraping of the squamocolumnar junction of the cervical epithelium with a wooden spatula. Specimens were mixed on one side of a glass slide and fixed with a spray fixative. On arrival to the cytopathology laboratory at the Health Sciences Centre, smears were stained by the standard Papanicolaou method. Smears were classified by standard criteria into normal, benign atypia (inflammatory, reparative), or dysplastic atypia. The degree of atypia (slight, moderate, severe, cardinoma in situ, or suggestive of

Table V. Microbiology of vagina

	Controls		Cases with cervical neoplasia		
	No.	%	No.	%	p
Gardnerella vaginalis	18/54	33	15/32	47	0.21
Ureaplasma urealyticum	30/53	57	15/31	48	0.47
Mycoplasma hominis	13/52	25	11/30	37	0.26
Trichomonas vaginalis	1/52	2	1/32	3	0.73
Candida albicans	8/51	16	3/31	10	0.44
Other yeast	4/50	8	2/30	7	0.83

Table VI. Association of human papilloma virus with cervical neoplasia after stratification by sexual experience

	No. with human papilloma virus		
No. of lifetime sexual partners	Controls	Cases with cervical neoplasia	p
1-4	1/27	8/15	0.0007
5-9	1/11	0/3	—
≥10	0/15	5/13	0.03

invasion) was based on the degree of nuclear and cytoplasmic changes.

Histopathology. Cervical tissue was fixed in 10% phosphate-buffered formalin, processed and blocked in paraffin, and sectioned at a 4 to 5 μm thickness. These sections were then stained with hematoxylin and eosin and, when necessary, by periodic acid–Schiff reaction with or without diastase. The degree of cervical intraepithelial neoplasia was graded as slight dysplasia (grade I), moderate dysplasia (grade II), severe dysplasia or carcinoma in situ (grade III). The presence or absence of microinvasion was noted.

Since human papilloma virus cannot be cultivated in vitro, we accepted as minimum evidence of its presence the appearance of koilocytosis in either or both cytologic smears or biopsy. In addition to koilocytosis, dyskeratosis and basal cell hyperplasia were commonly seen in specimens labeled as human papilloma virus.[7]

Data analysis. Two-tailed Student's *t* test was used for comparison of mean, and χ^2 test or one-tailed Fisher's exact test was used for comparison of proportions where appropriate.[8]

Results

Demographic data. Referred women with cervical intraepithelial neoplasia were older (27.0 ± 12.8 vs. 21.9 ± 4.0, p = 0.035) and more often not single (61% vs. 76%, p = 0.05) than were control women. Both groups were of comparable socioeconomic status (Table I). Control women had significantly fewer pregnancies than case women (1.2 ± 1.4 vs. 2.8 ± 2.5, p = 0.001), which was an expected difference, since controls represented women attending for contraceptive advice. Cases and controls did not significantly differ at the age of initiation of sexual intercourse (15.4 ± 3.5 vs. 16.2 ± 2.1, p = 0.23); however, cases had significantly more lifetime sexual partners (15.6 ± 23.7) than did controls (6.6 ± 6.7, p < 0.04) (Table I).

Clinical data. No cases or controls had vulvar condyloma acuminata or genital ulcers detected at the time of enrollment. Bacterial vaginosis was present in 45% of cases and 29% of controls (p = 0.1). Mean vaginal pH was 4.81 ± 0.41 in cases and 4.66 ± 0.51 in controls (p = 0.15) (Table II). Cervical mucopus was identified in 14% of controls and 13% of cases. Cervical mucus contained ≥10 polymorphonuclear leukocytes per × 1000 field in 20% of controls and in 39% of cases. None of these differences were significant, and thus neither bacterial vaginosis nor mocupurulent cervicitis were found significantly more often in cases than in controls. Although mucopus was not associated with cervical intraepithelial neoplasia, it was highly suggestive of *C. trachomatis* infection, since six of 15 women with *C. trachomatis* recovered had mucopus versus eight of 87 women who were culture negative (p = 0.0005).

Colposcopic findings. The area of the cervix occupied by the transformation zone was estimated as a percentage of the total area of the portio in cases and controls. The transformation zone was significantly larger among cases than controls (30.0% ± 6.6% vs. 24.9% ± 8.3%, p = 0.0003).

As expected, colposcopic changes were highly suggestive of cervical intraepithelial neoplasia, since 67% of cases versus 12% of controls had white epithelium and/or keratosis observed (p < 0.0001). Similarly, 30% of the cases versus 2% of the controls had mosaicism and/or punctation (p < 0.0001). Atypical vessels were found in one case of grade III cervical intraepithelial neoplasia. All cases had an atypical transformation zone compared to 14% of the controls (p < 0.001) (Table III).

Colposcopic changes were also useful in predicting human papilloma virus infection. Colposcopic findings

Table VII. Association of vaginal flora types with cervical neoplasia after stratification by sexual experience

No. of lifetime sexual partners	*Controls*			*Cases with cervical neoplasia*			*p*
	Vaginal flora			*Vaginal flora*			
	I	*II*	*III*	*I*	*II*	*III*	
1-4	20	6	1	6	6	2	0.04
5-9	4	4	0	1	2	0	0.06
≥10	7	8	0	3	6	4	0.03

suggestive of human papilloma virus were white shiny lesions with an irregular surface and no characteristic vascular pattern.[9] In 17 patients with human papilloma virus diagnosed by biopsy and/or cytology, the colposcopic findings suggested this diagnosis in 15 (sensitivity of 88%). Of 71 patients without cytologic and/or biopsy evidence of human papilloma virus, 55 lacked colposcopic evidence of the infection (specificity of 77%). Thus the predictive value of positive colposcopic findings for human papilloma virus was 48% and predictive value of negative findings was 96%.

Microbiologic data

Cervix. C. trachomatis was the most frequently recovered pathogen from the cervix, having been isolated from 13% of controls and 15% of cases. *N. gonorrhoeae*, herpes simplex virus, and cytomegalovirus were all recovered less frequently. There were no significant differences between groups in the prevalence of infection with any of these agents. Although not diagnosed by conventional microbiologic methods, human papilloma virus was seen in pathologic specimens significantly more often in cases (45%) than in controls (4%, $p < 0.0001$) (Table IV).

Vagina. The isolation rates for *G. vaginalis, U. urealyticum, M. hominis, T. vaginalis, C. albicans,* and other yeast found in the vagina were not statistically significantly different between cases and controls (Table V).

Relationship between cervical intraepithelial neoplasia and vaginal flora as assessed by Gram stain. Vaginal flora was graded according to criteria defined in section on methods; 33 of 52 controls and 11 of 31 cervical intraepithelial neoplasia cases had a grade 1 flora ($p < 0.01$), whereas grade 3 vaginal flora was present in one of the 52 controls and six of 31 cervical intraepithelial neoplasia cases ($p < 0.02$) (see Table II). Flora with predominant lactobacilli was present in 26 of 52 controls and 10 of 31 cervical intraepithelial neoplasia cases ($p = 0.09$). Gram stain of vaginal secretions was suggestive of bacterial vaginosis in 15 of 52 controls and 14 of 31 cases ($p = 0.1$). Thus vaginal flora was significantly different between cases and controls, and this difference could not be entirely explained by an increased prevalence of bacterial vaginosis.

Association between cervical intraepithelial neoplasia and human papilloma virus infection and vaginal flora types after stratification by sexual experience. Since women with cervical intraepithelial neoplasia were more sexually experienced than women in the control group and since the disease pathogens studied are sexually transmitted, a causal association between cervical intraepithelial neoplasia and a pathogen would be strengthened if the association were independent of sexual experience. Women were stratified by sexual experience into three groups: those with 1 to 4 lifetime sexual partners, those with 5 to 9, and those with 10 or more (Tables VI and VII). Both human papilloma virus infection and altered vaginal flora remained significantly associated with cervical intraepithelial neoplasia independent of sexual experience.

Comment

The significant findings of this study are that these women with cervical intraepithelial neoplasia had (1) more sexual partners, (2) a larger transformation zone, (3) a higher prevalence of human papilloma virus infection, and (4) an alteration in vaginal flora more often than controls. Cases and controls were comparable in terms of socioeconomic status, and age at first sexual intercourse, but they significantly differed in terms of age, gravidity, and marital status.

Our observation that women with cervical neoplasia have significantly larger numbers of lifetime sexual partners confirms what has been reported before[1] and underscores the important influence of sexual behavior as a risk factor for cervical intraepithelial neoplasia. All of our cases were sexually active.

Aside from possessing distinctive features of the transformation zone which suggested neoplasia, our cases also had a significantly larger overall area of the transformation zone. This is of particular interest, since it is within the transformation zone that the dysplastic process arises. The presence of a larger transformation may be a risk factor for cervical neoplasia, since this could provide a larger "target area" for an initiator or promoter to act. Whether women with a larger area of transformation zone are at an increased risk of cervical intraepithelial neoplasia will require prospective study in a larger population in order to be confirmed.

Although we examined cases and controls for a variety of sexually transmitted pathogens, only human papilloma virus was found to be present significantly more commonly in cases than in controls. This association was independent of sexual behavior. This finding adds further support to the growing body of evidence that links epithelial neoplasia with papilloma virus infection. It is important to point out that we diagnosed papilloma virus infection entirely by its cytopathic effect. Since we know neither the sensitivity nor the specificity of this effect, we may be underdiagnosing or overdiagnosing its presence. Improved detection of human papilloma virus infection must await the availability of suitable human papilloma virus–DNA probes for in situ hybridization.

It is noteworthy that *C. trachomatis* was isolated commonly (13% to 15%) from cases and controls and was isolated approximately six times more frequently than *N. gonorrhoeae*. Although previous studies have suggested that *C. trachomatis* is isolated more frequently from women with cervical intraepithelial neoplasia and that atypical changes are commonly seen in women with chlamydial infection,[10] no association of *C. trachomatis* infection with cervical intraepithelial neoplasia was found despite a high prevalence of infection in our patient population. An important limitation in our study design is that we sought current infection. What may be more important in a disease such as cervical intraepithelial neoplasia, which can take years to evolve, may be infection history. Prior exposure to herpes simplex virus, cytomegalovirus and *C. trachomatis* may be best assessed by serologic tests that have yet to be done on this sample of women.

Finally, we found that the distribution of vaginal flora types as assessed by Gram stain was significantly different in cases and controls. Grade I vaginal smears demonstrated a homogenous gram-positive flora and were found in 69% of the controls and 33% of the cases ($p = 0.01$), whereas a heterogenous bacterial morphotype flora was seen significantly more often in cases (67%) than in controls (31%, $p = 0.01$). The heterogenous flora was usually but not exclusively typical of that seen with bacterial vaginosis. Bacterial vaginosis was seen more commonly in cases (45%) than controls (29%), but this was not statistically significant ($p = 0.1$). Grade III smears that demonstrated exclusively gram-negative organisms (cocci, rods) without morphotypes typical of *G. vaginalis* were seen almost exclusively in cases; the bacteria associated with these flora patterns have yet to be defined, and thus this association needs to be cautiously interpreted. However, the association of altered vaginal microbial flora and human papilloma virus with cervical intraepithelial neoplasia suggests that a synergistic interaction between this altered flora and human papilloma virus could result in cervical intraepithelial neoplasia as has been hypothesized for human papilloma virus and herpes simplex virus.[11]

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A complete list of references will be supplied to readers upon request.

A prospective study of blood pressure in pregnancy: Prediction of preeclampsia

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A prospective study of blood pressure recording was conducted in 1000 patients, at each antenatal visit, with the use of an automatic random-zero sphygmomanometer. In 46 patients, among 808 primigravid women, who developed preeclampsia, the diastolic and mean blood pressures were significantly elevated compared to values at the first antenatal visit ($p < 0.01$, 9 to 12 weeks). This difference was sustained throughout pregnancy until delivery by at least 10 mm Hg as compared to pressures in the normotensive group. Sensitivity for predicting preeclampsia early in pregnancy with an elevated blood pressure measurement (130 to 135/80 to 85 mm Hg) ranged from 16% to 57% while specificity ranged from 75% to 98%. The results substantiate an early vasospasm (9 to 12 weeks) in those women destined to develop preeclampsia. (AM J OBSTET GYNECOL 1985;151:191-6.)

Key words: Pregnancy-induced hypertension, chronic hypertension, prediction of preeclampsia

Several blood pressure studies during the antenatal period have showed a decrease in blood pressure between 16 and 20 weeks.[1] Gallery et al.[2] demonstrated that pregnancy-induced hypertension could be predicted by a significant increase in blood pressure as early as 17 to 20 weeks. This significant difference was observed with a standardized random-zero sphygmomanometer but was not apparent with the usual sphygmomanometer.[2] However, the sample size of that study was small and there was no discrimination between primigravid women, who are more prone to preeclampsia, and multiparous women, in which chronic hypertension is more prevalent. Recently, another group[3] showed that a mean arterial blood pressure of 90 mm Hg or more between 18 and 26 weeks was predictive of preeclampsia.

In a preliminary study[4] on 366 pregnant women, we observed a similar decrease of blood pressure from 13 to 21 weeks, in systolic and diastolic blood pressure in women who remained normotensive throughout pregnancy, together with a significant rise in systolic and diastolic pressure as early as 13 to 16 weeks in 14 primigravid women who later developed preeclampsia. More observations were needed to confirm these findings, not only to show the constancy of the differences but also to establish whether preeclampsia could be predicted accurately with a marker, such as one blood pressure recording, early in pregnancy. Similar studies were also carried out with the mean blood pressure.

Material and methods

Population. Blood pressure was recorded in 1000 consecutive pregnancies, at each antenatal visit until delivery and at the postpartum visit, between November, 1979, and April, 1984. These women were cared for by a group of three private obstetricians and no intervention was suggested for any blood pressure reading. Seventeen patients were excluded (nine twin pregnancies and eight women who were not delivered at the institution). Thus the results included 983 women (808 primigravid, 175 multiparous) with a singleton pregnancy who were delivered at Notre-Dame Hospital. More than 7800 blood pressure recordings (in duplicate) were analyzed. Mean maternal age was 25.5 ± 4.4 years (±SD). Mean gestational age at delivery was 39.4 ± 1.7 weeks (±SD). The cesarean section rate was 14.8%.

Methods. Arterial blood pressure was taken from the right arm with the patient in a sitting position, after a minimum of 5 minutes of rest. This measure was taken twice at an interval of 2 minutes. If a discrepancy occurred between the two readings, then blood pressure was taken again until similar readings were observed (difference of <5 mm Hg). Then the last two recordings were kept for analysis. A nurse was responsible for the procedure and for daily calibration of the sphygmomanometer.

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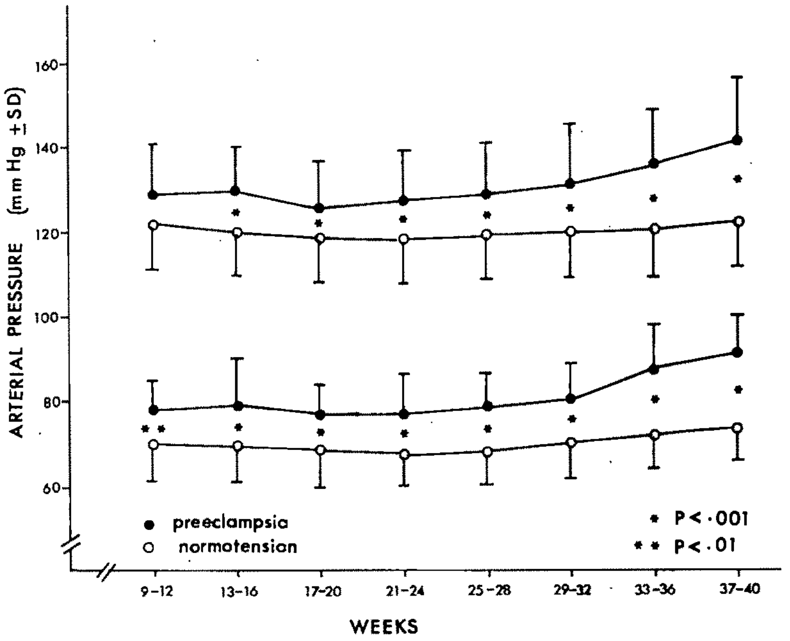

Fig. 1. Averaged systolic and diastolic arterial pressures (± 1 SD) in primigravid women who remained normotensive throughout pregnancy (o——o) and in those who developed preeclampsia (●——●) by periods of 4 weeks.

Table I. Incidence of hypertensive complications at delivery in primigravid and multiparous women

	Primigravid women		*Multiparous women*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*
No. of patients	808	–	175	–	983	–
Gestational hypertension	15	1.9	5	–	20	2.0
Preeclampsia-eclampsia						
Mild, moderate	42	5.2	1	–	43	–
Severe	1	–	2	–	3	–
Eclampsia	1	–	0	–	1	–
Hepatic*	2	–	0	–	2	–
Total	46	–	3	–	49	5.0
Chronic hypertension						
Without preeclampsia	31	3.8	21	12.0	52	5.3
With preeclampsia	6	–	2	–	8	–
Total	37	4.5	23	–	60	6.1

*Hepatocellular damage evidenced by altered biochemical tests, thrombocytopenia, and jaundice.

The apparatus was an automatic random-zero sphygmomanometer with digital display (Dinamap 845*) which is based on the oscillometric principle (same principle as the usual sphygmomanometer). One advantage of this apparatus is its proficiency to decrease acoustic and physical artifacts. The diastolic reading corresponded to phase IV of Korotkoff sounds. The accuracy of blood pressure measurement was validated by the obtaining of similar values (in the range of 160 mm Hg systolic to 50 mm Hg diastolic) of intra-arterial radial pressure during surgical procedures. Mean arterial pressure was calculated according to the formula: Systolic blood pressure plus twice diastolic blood pressure/3.[3]

Data were stored at the calculus center of the University of Montreal and analyzed with the Statistical Package for the Social Sciences. Comparisons between differences were analyzed with the unpaired t test,[5] while sensitivity, specificity, and predictive values were assessed by the method of Vecchio.[6]

Results

The perinatal mortality rate was 4.1/1000 births. There were two stillbirths and two neonatal deaths (one due to congenital cardiopathy) thus providing a corrected perinatal mortality rate of 3.1/1000 births.

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*Bionetics, California.

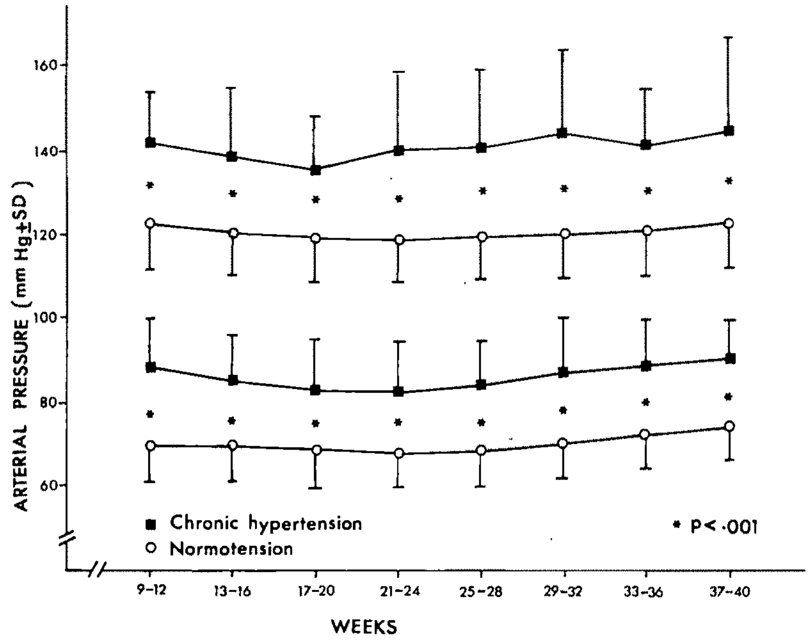

Fig. 2. Averaged systolic and diastolic arterial pressures (±1 SD) in multiparous women who remained normotensive throughout pregnancy (o——o) and in those who developed chronic hypertension (■——■) by periods of 4 weeks.

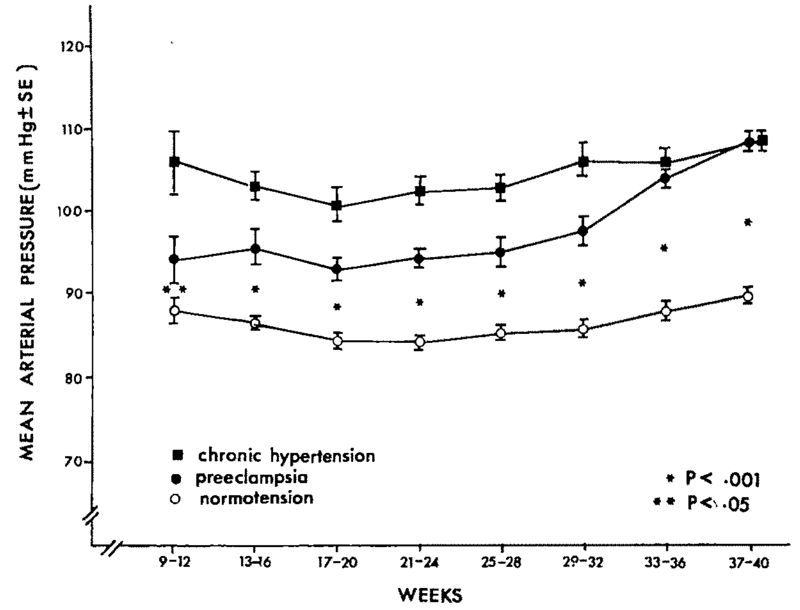

Fig. 3. Averaged mean arterial blood pressures (±1 SE) in women who remained normotensive throughout pregnancy (o——o), in women who developed preeclampsia (●——●), and in women who developed hypertension (■——■) by periods of 4 weeks.

Hypertensive complications are illustrated in Table I. Gestational hypertension was diagnosed when hypertension alone (≥140/90 mm Hg) occurred after 20 weeks and disappeared at the postpartum visit. The diagnosis of preeclampsia was ascertained by the presence of hypertension ≥140/90 mm Hg on two occasions 6 hours apart, edema (>1 kg/wk) or proteinuria (Albustix 1+) on two occasions 6 hours apart after 20 weeks, elevated serum urate levels (4.6 mg/dl or more), and a blood pressure of ≤120/80 mm Hg at 6 weeks post partum. Chronic hypertension was defined by a prepregnancy personal history of hypertension or persistence of hypertension 6 weeks post partum.

Averaged systolic and diastolic blood pressures

Table II. Sensitivity, specificity, and positive and negative predictive values (percentages) of recordings of 130 and 135 mm Hg systolic blood pressure as predictors of preeclampsia

Weeks' gestation	*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*
130 mm Hg				
9-12	46.2	75.4	12.5	94.9
13-16	57.6	81.2	16.0	96.9
17-20	43.3	85.3	15.1	96.1
21-24	41.2	86.2	14.9	96.2
25-28	42.1	82.7	13.1	95.8
135 mm Hg				
9-12	30.8	87.1	15.4	94.3
13-16	42.4	90.2	21.2	96.2
17-20	18.9	93.5	14.9	95.0
21-24	29.4	92.8	19.2	95.7
25-28	28.9	91.4	17.2	95.4

Table III. Sensitivity, specificity, and positive and negative predictive values (percentages) of recordings of 80 and 85 mm Hg diastolic blood pressure as predictors of preeclampsia

Weeks' gestation	*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*
80 mm Hg				
9-12	46.2	84.2	18.2	95.4
13-16	45.5	89.8	21.7	96.4
17-20	32.4	91.0	17.9	95.7
21-24	38.2	93.4	25.5	96.3
25-28	44.7	91.7	25.0	96.4
85 mm Hg				
9-12	30.8	93.0	25.0	94.6
13-16	30.3	96.6	35.7	95.7
17-20	16.2	97.4	27.3	95.1
21-24	20.2	97.9	36.3	95.5
25-28	18.4	98.0	36.8	95.1

(±SD) by 4-week periods in the group of normotensive nulliparous women were compared to the blood pressures in the group of nulliparous women who later developed preeclampsia (Fig. 1). From 13 weeks onward, a significant difference in systolic pressure was observed between the two groups; the increase ranged from 7 to 22 mm Hg in the group destined to develop preeclampsia ($p < 0.001$, Fig. 1). From the first antenatal visit (9 to 12 weeks), the diastolic pressure was consistently more elevated in patients eventually developing preeclampsia; elevations ranged from 7 mm Hg ($p < 0.01$, at 9 to 12 weeks) to 10 to 15 mm Hg until delivery ($p < 0.001$, Fig. 1).

In the multiparous women, averaged systolic and diastolic blood pressures (±SD) showed, as expected, a significant increase of about 20 mm Hg systolic and 15 to 17 mm Hg diastolic throughout pregnancy in women with chronic hypertension compared to normotensive multiparous women ($p < 0.001$, Fig. 2).

Comparisons of mean arterial pressures (±SE) between patients eventually developing preeclampsia, patients with chronic hypertension, and normotensive patients showed significant increases in both women with eventual preeclampsia and those with chronic hypertension compared to the normotensive group ($p < 0.05$, 9 to 12 weeks, preeclampsia; $p < 0.001$, all other periods of pregnancy; Fig. 3). The difference in excess of mean blood pressure was 10 mm Hg in patients with eventual preeclampsia and 16 to 20 mm Hg in patients with chronic hypertension as compared to the normotensive patients at any period of pregnancy (Fig. 3).

Although significant differences in blood pressure were clearly established in the group of women with eventual preeclampsia, such a prediction needed to be substantiated in individual patients. Clinical markers of easy access to identify early in pregnancy those patients who will develop preeclampsia are yet to be found. Single values of blood pressure (systolic, diastolic, mean) were tested as potential predictors of preeclampsia in the first five periods of observations (9 to 12, 13 to 16, 17 to 20, 21 to 24, and 25 to 28 weeks); these values were examined with the method of Vecchio.[6]

With a systolic value of 130 mm Hg, the sensitivity

Table IV. Sensitivity, specificity, and positive and negative predictive values (percentages) of mean blood pressures of 85 and 90 mm Hg as predictors of preeclampsia

Weeks' gestation	*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*
85 mm Hg				
9-12	84.6	38.6	9.5	97.1
13-16	81.8	43.2	8.2	97.5
17-20	86.5	50.4	9.5	98.4
21-24	85.3	49.7	9.0	98.3
25-28	86.8	48.9	9.5	98.4
90 mm Hg				
9-12	61.5	62.0	11.0	95.5
13-16	69.7	66.4	11.4	97.3
17-20	67.6	75.4	14.2	97.5
21-24	55.9	78.1	13.0	96.8
25-28	71.1	73.9	14.4	97.6

was around 50% and specificity was about 80%. At a value of 135 mm Hg, the number of eventually preeclamptic patients who exhibited such systolic pressure was small (18% to 42%) while as few as 6.5% to 12.9% of women who remained normotensive had such systolic pressure (Table II). The positive predictive value for 130 and 135 mm Hg was rather low, as expected,[6] because only 5% of the population studied had the disease; conversely, the negative predictive value was 94% to 96% (Table II) because the fraction of the population not having preeclampsia was large.[6]

When diastolic values were examined as predictors of preeclampsia, sensitivity was between 38% and 46% at 80 mm Hg and specificity ranged from 84% to 98% at 80 and 85 mm Hg (Table III). Here again positive and negative predictive values yielded percentages similar to those with systolic pressure (Table III). The mean arterial pressure showed an improved sensitivity (82% to 87%) with a value of only 85 mm Hg but specificity was <50% in most instances (Table IV). Specificity increased somewhat at a value of 90 mm Hg but the sensitivity was then decreased because several eventually preeclamptic women did not have a mean blood pressure of ≥90 mm Hg before 29 weeks (Table IV).

Comment

A cohort of private patients with non–high-risk pregnancies yielded 5% preeclampsia and 6% chronic hypertension. This incidence of chronic hypertension is a reflection of a rather older population (up to 40 years of age). However, the frequency of superimposed preeclampsia was only 16% in primigravid women and 13% in the total sample, which is less than the reported frequency of 20% to 40% for this complication.

The fall in systolic and diastolic blood pressures between 13 and 25 weeks in both primigravid and multiparous women confirmed results in previous reports,[1,2] but a larger sample size permitted us to extend this fall during the entire second trimester rather than between 16 and 20 weeks.[2] Such a fall, coincident with increasing cardiac output and blood volume, illustrates a lowering of systemic vascular resistance in normotensive women. This study also confirms the increase of blood pressure in those women destined to develop preeclampsia. However, recording from the first antenatal visit has demonstrated for the first time a significant increase in diastolic pressure as early as 9 to 12 weeks rather than at 17 to 20 weeks.[2] This latter observation suggests a potential to identify from the first antenatal visit, in the first trimester, those women destined to develop preeclampsia with a mean diastolic blood pressure value of as low as 77 mm Hg or with a mean arterial blood pressure of 93 mm Hg. In addition, this finding may be of some help for the clinician so that a particular pregnancy may be observed more closely and other tests may be carried out to confirm future preeclampsia.[7] More important, the increase in diastolic blood pressure suggests, for the first time, an early vasospasm at 9 to 12 weeks. Such patients did not have chronic hypertension because the blood pressure was <140/90 mm Hg before 20 weeks, the well-defined criteria of preeclampsia were present, the blood pressure returned to normal at 6 weeks postpartum, and also because the reported incidence of preeclampsia of 5% in this study was not different from the incidence of preeclampsia in our institution. Such an early vasospasm favors early vascular pathophysiologic changes because of the presence of the conceptus in the first trimester.

Another objective was to identify predictors of preeclampsia from a cohort of pregnant women. Single values of systolic, diastolic, and mean arterial pressures were tested to ascertain their potential as markers for future preeclampsia.

The sensitivity and specificity for diastolic values of 80 and 85 mm Hg were not different from those of previous preliminary reports.[4] The rather poor sensitivity suggests that the systemic manifestation of vasospasm does not appear at the same period in every woman eventually developing preeclampsia and that its expression is not necessarily evident at rest, in a sitting position. The specificity, however, increases with increasing values of both systolic and diastolic blood pressures. Percentages of specificity are similar to those previously reported.[4] Predictive positive values were higher in a preliminary report[4] than in the present study; this can be attributed to the larger sample size.[6] With the mean arterial pressure, the sensitivity and specificity approached those reported by others.[3] However, the predictive positive value was less in the present study because the sample size was larger than the 85 patients reported by Oney and Kaulhausen.[3]

From this information, clinical evaluation of blood pressure with an automatic random-zero sphygmomanometer will identify a subgroup of patients at potentially high risk of developing preeclampsia. Concurrently, a diastolic blood pressure <85 mm Hg as early as 9 to 12 weeks has a 95% chance to predict normotension throughout pregnancy. Only 2% of women with that diastolic blood pressure in the second trimester will develop preeclampsia; this enables the identification of the low-risk group in a cohort of pregnant women.

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Minibolus diazoxide in the management of severe hypertension in pregnancy

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Severe hypertension poses immediate and potentially lethal maternal risks. Prompt reduction in blood pressure should be an urgent priority, with concomitant anticonvulsant therapy. Previous reports confirm intravenous diazoxide to be an effective agent. Concern exists over adverse reactions associated with bolus injection of the standard 300 mg dose. Many physicians are unaware that diazoxide may be given in increments. This study describes the use of diazoxide in 34 patients with severe preeclampsia. They received minibolus injections of 30 mg every 1 to 2 minutes. The maximum dose required was 150 mg and no maternal hypotension occurred. Maternal side effects were minimal and no cases of fetal distress occurred; a perinatal mortality was zero. It appears that minibolus diazoxide rapidly lowers maternal blood pressure without the adverse effects described with a standard bolus dose. Its advantages are contrasted with the limitations of conventional hydralazine therapy. (AM J OBSTET GYNECOL 1985; 151:196-200.)

Key words: Minibolus diazoxide, severe hypertension

Severe preeclampsia or pregnancy-induced hypertension should be considered as a form of malignant (accelerated) hypertension. In previously normotensive patients rapidly progressive hypertension can cause vascular damage and hypertensive encephalopathy at lower blood pressures than in chronic hypertensive pa-

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tients. In preeclampsia prompt reduction of blood pressure is essential to minimize vascular injury and prevent cerebrovascular hemorrhage, which is the leading cause of maternal death in eclampsia. The specific therapy for acute hypertension in pregnancy, however, is still controversial. Many physicians avoid using antihypertensive agents even in severe pregnancy-induced hypertension and rely on anticonvulsant drugs, such as magnesium sulfate, that have no significant hypotensive effect.

Placental ischemia and generalized vasoconstriction are prominent pathophysiologic features of pregnancy-induced hypertension. Theoretically, peripheral vasodilator drugs are the ideal agents to lower blood pressure, and intravenous hydralazine is widely used in the treatment of severe preeclampsia. Diazoxide, another potent vasodilator, was thought to be effective only when administered rapidly as a 300 mg intravenous bolus. Reports of fetal distress[1-3] and maternal death[4, 5] from profound hypotension have curtailed its use in obstetrics. Redman[6] found that giving the drug in small 30 mg increments to pregnant patients was effective in lowering blood pressure. Other reports confirm the finding that by using a smaller dose[7] or by giving it in a slow intravenous infusion[8] the blood pressure can be lowered without the precipitate hypotension that may complicate the standard bolus dose. This study describes the efficacy and safety of minibolus diazoxide in 34 patients with severe pregnancy-induced hypertension. Results suggest that it is a useful agent and its advantages over conventional hydralazine therapy are discussed.

Patients and methods

Thirty-four patients with a blood pressure >160/115 mm Hg or a mean arterial pressure >130 torr were admitted to the study. No patients, including eight with preexisting mild essential hypertension, were receiving antihypertensive medication. Thirty-two (94.1%) were in the gestational range of 27 to 34 weeks of gestation and the remainder were at term.

All patients received 5 gm of magnesium sulfate by intravenous injection shortly after admission. Anticonvulsant therapy was maintained by intravenous infusion of 2 gm/hr. Magnesium levels were measured every 2 to 4 hours and the rate of infusion was adjusted to maintain blood levels in the range of 2 to 2.8 mmol/L. Blood pressure readings were monitored continuously by a cardiac monitor.* Minibolus diazoxide, 30 mg given intravenously for a period of 30 to 60 seconds, was administered every 1 to 2 minutes until diastolic levels <90 torr were obtained. Blood pressure readings were recorded from the cardiac monitor every 5 minutes for 30 minutes and thereafter every 15 minutes.

Maternal hemoglobin, hematocrit, electrolytes, uric acid, glucose, blood urea nitrogen, and creatinine levels were determined prior to and 1 hour after diazoxide treatment, and measurements were repeated every 6 hours for 24 hours. Platelet counts, serum fibrogen, prothrombin, and partial thromboplastin time were measured before treatment, and measurements were repeated every 6 hours. Real-time ultrasound scans were performed to confirm fetal presentation, assess amniotic fluid volume, and rule out congenital anomalies. Continuous electronic fetal heart rate (FHR) monitoring was instituted with an external technique on a Corometrics 112 fetal monitor. Urine output was measured on an hourly basis and maternal fluid therapy was adjusted according to central venous pressure readings. Neonatology consultations were obtained and the route of delivery was chosen on the basis of obstetric factors. Induction of labor with intravenous oxytocin was started in four patients. All neonates were transferred to an intensive care nursery. Cord blood and neonatal samples were analyzed for electrolytes, plasma glucose, hemoglobin, platelet, blood gases, and bilirubin levels.

The mean and SD were calculated for each variable. Differences for each parameter were evaluated by a paired t test at $p < 0.02$.

Results

The average dose required to attain a mean arterial pressure of <95 torr was 120 mg (range, 90 to 150 mg). This effect was observed in all 34 patients within 5 to 10 minutes. No excessive hypotension occurred since the lowest blood pressure recorded was 105/70 mm Hg (mean arterial pressure approximately 80 torr). The mean duration of effect was 4.8 ± 3.7 hours. One patient required a second treatment in 1 hour and three others prior to delivery. All patients were delivered within 6 hours (mean, 3.2). The cesarean section rate was 88.2%.

Fourteen of 34 patients experienced nausea or vomiting during initial magnesium sulfate injection, but these effects were not noted during or after diazoxide therapy. There were no significant changes in coagulation parameters, blood urea nitrogen, or uric acid levels within 24 hours after treatment. Pretreatment plasma urate levels were elevated in all patients (range, 410 to 608 μmol/L). Mean hematocrit values showed a decrease from 0.43 to 0.39 but were not statistically significant. The mean plasma glucose level rose from 4.6 ± 1.1 to 5.8 ± 1.8 mmol/L and the plasma sodium level increased slightly from a mean of 135 ± 5.1 to

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*Siemens Sirecust 400 Series, Model No. 404, Iselin, New Jersey.

Table I. Infant population according to birth weight

Birth weight (gm)	*No. of infants*
500-999	1
1000-1499	9
1500-1999	19
2000-2499	2
>2500	3

Table II. Neonatal complications*

Complication	*No. of cases*	*Rate (%)*
Respiratory distress syndrome	14	41.1
Hypoglycemia	8	23.5
Necrotizing enterocolitis	1	2.9
Hyperbilirubinemia	6	17.6
Hyperkalemia	1	2.9

*Fifteen (44%) of 34 neonates had one or more of the above complications.

138.5 ± 6.8 mmol/L. Neither difference was statistically significant. Serum creatinine levels showed a significant but reversible change from a mean of 98 ± 35 to 130 ± 37.8 mmol/L ($p = 0.02$). There were no instances of fetal distress (bradycardia or late decelerations). Intermittent variable decelerations were noted in 16 patients, 13 of whom were assessed by ultrasound as having severe oligohydramnios. Decreased FHR variability was noted in eight (23.5%) of 34 tracings, all in patients of a gestational age <31 weeks.

Table I summarizes the infant population distributed according to birth weight. No perinatal deaths occurred, but neonatal complications were observed in 44.1% of infants including cases of respiratory distress syndrome, hypoglycemia, necrotizing enterocolitis, hyperbilirubinemia, and hyperkalemia. Neonatal hyperglycemia previously described with maternal diazoxide therapy was not noted in our series since the mean glucose level was 3.1 mmol/L. Eight infants had hypoglycemia attributable to extreme prematurity and none had a glucose level >5 mmol/L. No cases of neonatal hemorrhage or thrombocytopenia were recorded. Six infants had bilirubin levels >170 µmol/L and three received exchange transfusions. The study design did not allow for any evaluation of increased hyperbilirubinemia (because of displacement of bilirubin from albumin by diazoxide) but the observed incidence did not seem excessive. Table II lists the neonatal complications in the entire group.

Cord blood gas values of four infants showed a pH <7.2. All were interpreted as having respiratory acidosis on the basis of studies of base excess and Pco_2 levels. These four infants were from the group of 13 infants with severe oligohydramnios and variable decelerations in the FHR tracings. Thirty of 34 infants had a cord blood pH >7.2.

Ten neonates had 1-minute Apgar scores of <7 including six with scores <5 at 1 minute. Five infants had 5-minute Apgar scores of <7 but none showed any evidence of metabolic acidosis from cord blood gas analysis.

Comment

Severe pregnancy-induced hypertension poses immediate and potentially lethal maternal risks. Prompt reduction of blood pressure should be an equal and concomitant priority with anticonvulsant therapy. Considerable evidence justifies the urgency for immediate lowering of the blood pressure. In experimental animals acute hypertension produces segmental necrotizing arteriolitis at a mean arterial pressure >130 torr. The striking observation is the speed at which vascular damage occurs, being noted in 10 minutes and becoming extensive within 1 hour.[9] The malignant phase of hypertension may be due solely to this injury,[10] although humoral factors could also be contributory.[11] Pressure-forced dilatation of arterioles results in a loss of autoregulatory tone. Disturbed autoregulation of the cerebral circulation causes hypertensive encephalopathy and retinopathy. Severe pregnancy-induced hypertension and eclampsia demonstrate many of the features of malignant hypertension, although there are differences. The liver is a special target organ for the preeclamptic process, and cerebral edema is not a notable autopsy feature of eclampsia. The intimal hypertrophy of arteries observed in malignant hypertension is not evident in preeclampsia, yet the retinal arterioles demonstrate the alternating segments of constriction and dilatation typical of hypertensive injury.

In chronically hypertensive patients autoregulation of higher pressures is maintained because of gradual structural adaptation in the vessels. This protects them from cerebral injury at high pressures. Previously normotensive individuals have not experienced adaptation in this manner and have a lower threshold for forced vascular dilatation. Rapid increases in blood pressure may result in hypertensive encephalopathy or vascular damage at lower pressures. Hence the magnitude of blood pressure elevation is less useful than the recognition of the clinical syndrome. Conversely the shift in the autoregulation curve toward higher pressures means that patients with chronic hypertension do not tolerate lower perfusion pressures. Symptoms of cerebral hypoperfusion and cardiac or renal sequelae may develop if blood pressure is lowered either too rapidly or to normal levels. However, most patients with acute severe pregnancy-induced hypertension require urgent lowering of blood pressure, which may be carried out with minimal risk of harmful effects.

Diazoxide is both a potent peripheral vasodilator with immediate onset of action and a uterine relaxant. It is rapidly and extensively bound to serum proteins (>90%) and was thought to be effective only when administered by rapid injection of a 300 mg dose. The arteriolar receptor is unknown and the known pharmacokinetic characteristics do not explain why small doses given slowly are effective. Furthermore, the hypotensive action (up to 12 hours) often outlasts the brief period of high concentrations of the free drug. It may be that the binding to the arteriolar receptor is slowly reversible. The physiologic half-life of 3 to 12 hours also differs considerably from its biologic half-life of 28 ± 8.3 hours.

Finnerty[12] advocated the use of diazoxide in 1961 and reported his experience in 400 preeclamptic patients in 1971. Morris et al.[1] also concluded that the drug was a useful agent for the treatment of severe pregnancy-induced hypertension. Nevertheless, reports of serious maternal and fetal side effects have limited its use in obstetrics. Renal failure, myocardial infarction, and several maternal deaths have been reported.[4, 5] Anginal syndromes, symptoms of cerebral ischemia, hypotension,[13] and death in nonpregnant patients have also been described.[4] The occurrence of fetal bradycardia and fetal distress associated with severe maternal hypotension after diazoxide injection is cited as a reason prohibiting its use in pregnancy.[3] All of these reports concern the 300 mg intravenous bolus given either singly or in combination with diuretics or other antihypertensive agents.

Redman[6] described the use of the drug in small increments in 1977. Other reports of use of a smaller, 150 mg dose[7] and slow intravenous infusion of 15 mg/min[8] indicate significant efficacy without excessive fall in blood pressure. Results from our study confirm these findings and suggest further there are no harmful fetal or neonatal effects associated with minibolus administration. The drug does cross the placenta but is rapidly cleared from the fetus. Necrosis of the fetal pancreas has been described in animals in a study in which the doses used were several times higher than that used clinically.[14] In our study, as in others, glucose values in neonates showed no elevation.[15, 16] Prolonged use of diazoxide in human pregnancy causes no deleterious neonatal effects except for hypertrichosis, alopecia, and decreased bone age.[16]

Case reports in the literature of late decelerations occurring after the administration of diazoxide were associated with severe maternal hypotension. Animal data obtained by measuring uterine blood flow showed conflicting results in response to diazoxide injection. Studies demonstrating a decrease found that it occurs only after excessive hypotension develops[17] whereas another study showed no decrease in uteroplacental blood flow.[18] Moreover, changes in uterine blood flow do not necessarily correlate with changes in placental perfusion. The absence of abnormal FHR tracings in our study would contradict that any significant reduction in placental flow occurs. The decreased FHR variability in eight tracings could be explained by fetal immaturity as subsequent cord blood pH showed no cases of metabolic acidosis. The respiratory acidosis in four infants at birth was consistent with cord compression and variable decelerations.

Maternal hyperglycemia and sodium and water retention described with diazoxide therapy would seem from our results to be minimal after minibolus administration. The decreased protein concentration in pregnancy yields higher free-drug levels and lower amounts of protein-bound agent. This may explain why smaller doses given slowly lower BP with minimal side effects. It is known that the large amount of drug in the bound fraction may account for some of the toxic effects seen after high dosage or chronic administration.

The conventional approach to reducing BP in severe pregnancy-induced hypertension utilizes hydralazine. A 20 mg intravenous injection usually has an onset of action of 15 to 20 minutes but may be delayed for 30 to 40 minutes. When the drug is given by intravenous drip this delay is more likely and could be extremely hazardous to the patient with malignant hypertension. Most patients are already receiving intravenous infusions of magnesium sulfate and diazoxide therapy obviates the need for additional intravenous line. Hydralazine is also not compatible with dextrose solutions. It is not a uterine relaxant and studies measuring its effect on uterine blood flow have shown conflicting data. Hydralazine is metabolized by acetylation in the liver. The level of N-acetyl-transferase is genetically determined. Five percent of the population have decreased levels of the enzyme and these patients with slow acetylation will show an exaggerated response to the standard intravenous dose. Tachycardia, although rarely seen with diazoxide, is more common and troublesome with hydralazine. Headache, nausea, and vomiting cloud the clinical evaluation for impending eclampsia.

It appears that minibolus diazoxide rapidly lowers maternal blood pressure without the adverse effects described with a standard bolus dose. Diazoxide in any regimen should not be administered with diuretics or other antihypertensive agents. Binding to albumin may displace phenytoin and complicate its use in epileptic patients. Minibolus diazoxide provides an alternative to conventional hydralazine therapy for hypertensive crises such as severe pregnancy-induced hypertension.

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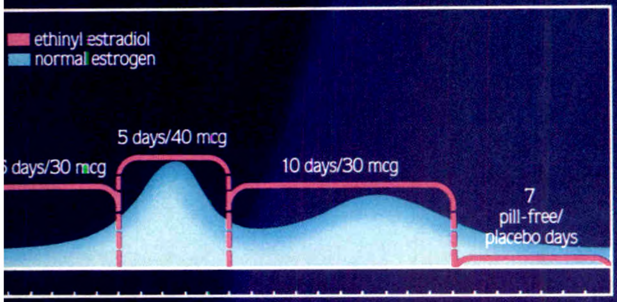

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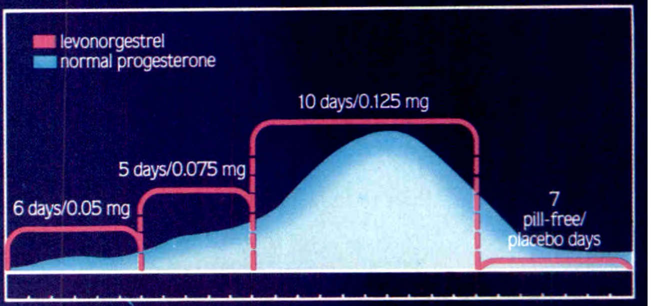

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Two separate 6-month studies demonstrate minimal impact on lipid levels; no statistically significant changes were observed in HDL, LDL, total cholesterol or triglycerides.[1,2] This is further supported by a separate 18-month prospective study in which 85 non-smoking women under the age of 35 were equally divided among 4 oral contraceptive formulations. With Triphasil, no statistically significant changes in total or HDL cholesterol occurred.[3] In this study, slight increases in triglycerides, which peaked at cycle 9, declined in subsequent cycles.

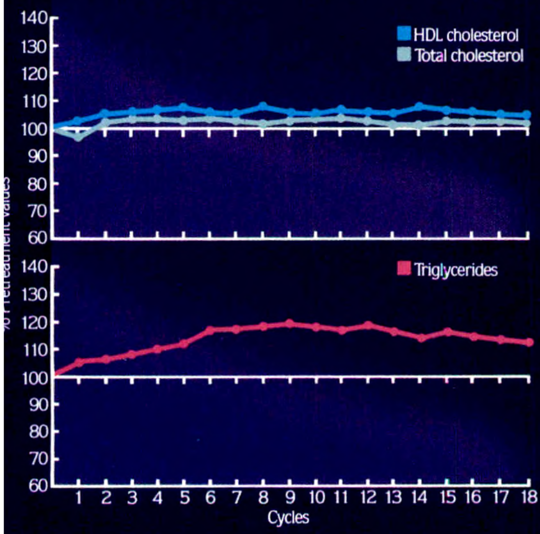

Figure 1: Monthly HDL cholesterol, triglycerides and total cholesterol levels for Triphasil over an 18-month period. (Adapted from Briggs.[3])

No significant impact on blood pressure means shown in 24-month study

In clinical trials over 2 years, Triphasil had no significant impact on blood pressure means. As shown in Figure 2, a high degree of stability was maintained with Triphasil compared to baseline.[2]

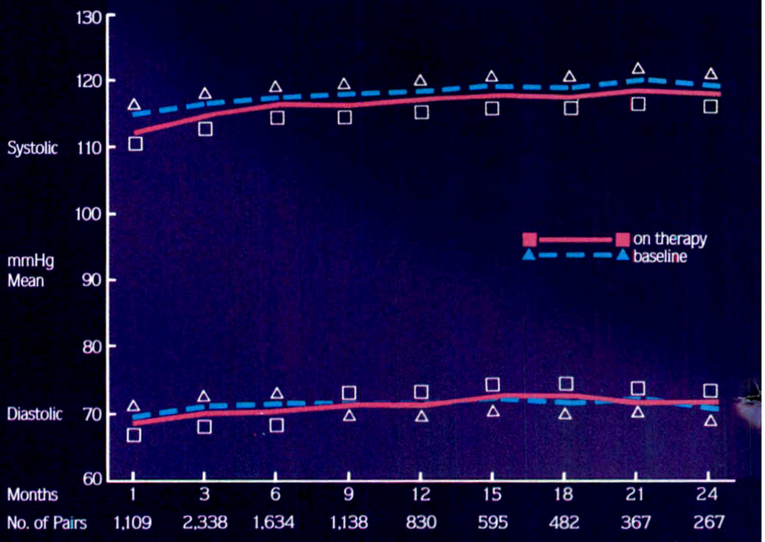

Figure 2: Blood pressure mean levels during treatment and baseline—systolic and diastolic.

you data this extensive

No significant impact on carbohydrate metabolism in 18-month study

Results at 6, 12, and 18 months demonstrate Triphasil has little influence on carbohydrate metabolism. Glucose tolerance and insulin levels generally remain within normal clinical limits. Figure 3 shows glucose tolerance test results from a study of 85 non-smoking women under the age of 35 who were equally divided among 4 oral contraceptive formulations. Blood samples were taken before Triphasil and at treatment cycles 6, 12, and 18. Findings at 18 months were not plotted because they were comparable to 12-month data.[3]

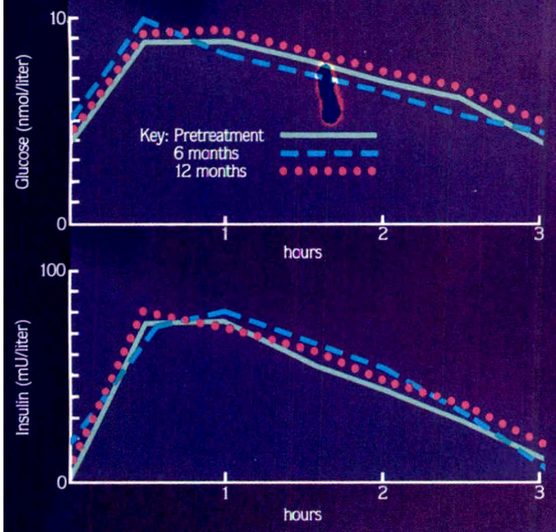

Figure 3: Oral glucose tolerance test results: Pretreatment and during treatment cycles 6 and 12.

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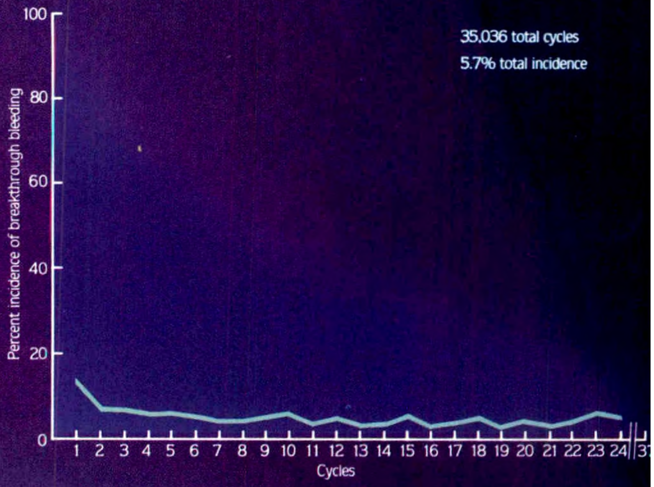

Figure 4: Incidence of breakthrough bleeding in 3,546 women during 24 months of Triphasil therapy.

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Unlike oral contraceptives which utilize a Sunday-start regimen, with Triphasil no additional protection was used during clinical studies in 3,546 women, and no pregnancies occurred during the first cycle. In worldwide post-marketing experience, there has been only one first cycle pregnancy reported in the literature.[4] For dosage and administration, please refer to brief summary.

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IN BRIEF:

TRIPHASIL*—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions. 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's.

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Anytime patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

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The treatment of grade 3 cervical intraepithelial neoplasia with cryotherapy: An 11-year experience

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The results of treatment of 453 patients with grade 3 cervical intraepithelial neoplasia by cryotherapy are presented. After excluding 31 patients who were lost to follow-up, the failure rate of primary treatment in the remaining 422 patients was 7.1% (30 patients). Outpatient re-treatment of those with persistent disease achieved an overall cure rate of 98%. Statistical analysis to determine prognostic significance with regard to persistent disease was carried out on the following variables: grade, size, and type of freeze. Type of freeze was the only variable to show statistical significance. Cryotherapy remains an effective outpatient modality for the treatment of grade 3 cervical neoplasia. A rigid protocol of patient selection and meticulous cryotherapy technique play a large role in obtaining the high cure rates reported in this study. (AM J OBSTET GYNECOL 1985;151:201-6.)

Key words: Cervical intraepithelial neoplasia, cryotherapy, colposcopy

Many papers have appeared since 1970 which review the treatment of cervical intraepithelial neoplasia with cryotherapy and laser techniques. Methods of patient evaluation, cryotherapy technique, and follow-up protocols have been outlined and standardized. Failure rates have been reported, reasons for these failures have been discussed, and alterations in patient evaluation and cryotherapy technique have been suggested and borne out in practice in an attempt to reduce the number of freeze failures.[1]

Of particular concern, however, is grade 3 cervical intraepithelial neoplasia. There is a suspiciously wide range of cryotherapy failure rates for grade 3 cervical intraepithelial neoplasia in the literature at the present time, e.g., from 8% to 9% (Benedet et al.[2]) to 38.8% (Ostergard[3]). A more detailed examination of this literature is found in Table I.

In this paper we report the results of an 11-year prospective trial of colposcopy, colposcopically directed biopsies, cryotherapy, and cytologic and histologic follow-up in 453 patients with grade 3 cervical intraepithelial neoplasia. In doing so, we wish to argue for the efficacy of this form of treatment for this lesion despite the recommendation that cryosurgery is no longer an appropriate or effective treatment for grade 3 cervical intraepithelial neoplasia.[3]

Material and methods

From January, 1972, to December, 1982, patients referred with an abnormal Papanicolaou smear were seen and evaluated at the abnormal cytology unit at Toronto General Hospital. At the initial visit a complete gynecologic and epidemologic history was obtained and a gynecologic examination performed. Cytologic testing, colposcopy, colposcopically directed biopsies, and pelvic examination were carried out on all patients. Endocervical curettage was carried out selectively on patients in whom endocervical disease could not be ruled out on colposcopic and endocervical speculum examination. Patients in whom the cervical intraepithelial neoplasia lesion was entirely visible on the neoplastic ectocervix and who had a negative endocervical speculum examination were not subjected to routine endocervical curettage in most cases. Treatment planning was done when the histologic diagnosis was confirmed. All biopsies were reviewed by Dr. D. W. Thompson, Department of Pathology, Toronto General Hospital. Patients with biopsy-proven severe dysplasia or carcinoma in situ were selected for cryotherapy and included in the study only if they met the following criteria: (1) entire lesion on the cervix was seen, (2) endocervical curettage was negative, (3) patient was not pregnant, (4) patient had not been exposed to diethylstilbestrol, (5) microinvasion or invasion was ruled out, (6) patient was interested in future childbearing, and (7) patient was reliable for close follow-up.

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Table I. The treatment of grade 3 cervical intraepithelial neoplasia with cryotherapy

Authors	*Year*	*Type* of neoplasia*	*No. of patients*	*Failure rate (%)*
Townsend and Ostergard	1971	SD	43	11.8
		CIS	19	10
Tredway	1972	CIN 3	49	30.6
Creasman et al.	1973	CIN 3	48	31.2
DiSaia, Townsend, and Morrow	1974	SD	107	9.3
		CIS	59	12
Creasman and Parker	1975	SD	88	27
		CIS	91	16
Gray and Christopherson	1975	SD (only)	10	20
Lickrish and Fortier	1977	SD	55	3.6
		CIS	53	17
Popkin	1978	SD	44	4
		CIS	31	9
Kaufman	1978	SD	98	17.3
		CIS	28	21.4
Walton	1980	CIN 3	95	31.6
Ostergard	1980	SD	28	7.1
		CIS	18	38.8
Hemmingsson	1981	CIS (only)	130	18
Wright	1981	CIN 3	44	25
Javameri	1981	SD	45	16
		CIS	15	15.3
Benedet	1981	SD	136	9
		CIS	229	8
Peckham	1982	SD	108	6.5
		CIS	35	20
Stuart	1982	SD	60	18
		CIS	4	0
Townsend and Richart	1983	CIN 3	53	10
Van Lent	1983	CIN 3	102	7
Creasman	1983	CIN 3	259	17.7
Present study	1984	CIN 3	422	7.1

*SD, Severe dysplasia; CIS, carcinoma in situ; CIN 3, grade 3 cervical intraepithelial neoplasia.

Cryotherapy was always performed within 2 months of treatment planning. Cryotherapy was done as an outpatient procedure, and no analgesia or anesthesia was necessary in most patients. Repeat colposcopy was done immediately prior to freezing in order to again record the margins of the transformation zone and the size of the lesion. Lesion sizes were divided between those occupying up to one quadrant (≤25% of the transformation zone) and those occupying greater than one quadrant. The Ohio Medical cryotherapy unit, with nitrous oxide as the refrigerant, was used with one of five different probes chosen according to the anatomic configuration of the cervix and the location and size of the lesion. The operating temperature of the probe was −75° C, and the pressure gauge was continuously checked to ensure adequate pressure with each freeze. A double freeze was performed in most patients. The cervix was frozen for 3 to 4 minutes, allowed to thaw for 5 to 7 minutes, then refrozen for a further 3 minutes. A single freeze of 3 to 4 minutes was used in a random fashion in 40 patients during 1979 and 1980 to determine its efficacy when compared to the double freeze. A 4 to 5 mm margin of freeze beyond the margins of the transformation zone was achieved in all patients. In large lesions multiple applications were often necessary, and cautery to the periphery of the transformation zone was added in a few patients. Following the procedure, patients were instructed to apply a vaginal antibiotic cream as long as the vaginal discharge lasted and to avoid tampons and sexual intercourse for 6 weeks. Follow-up visits were scheduled for 6 weeks and 3 months after treatment and then at 3- or 6-month intervals for the first 18 to 24 months depending on results of cytologic and colposcopic examinations. Yearly visits were scheduled thereafter.

A cryotherapy cure was defined as a patient followed for 1 year with at least two negative Papanicolaou smears. A freeze failure was defined as persistence if grade 2 or 3 cervical intraepithelial neoplasia was diagnosed on routine follow-up visits by Papanicolaou smears, biopsy, or endocervical curettage during the first 12 months following cryotherapy. Patients with grades 1, 2, or 3 cervical intraepithelial neoplasia occurring after 12 months following cryotherapy or after three negative Papanicolaou smears were regarded as having recurrent disease and were not considered as freeze failures. Patients showing only minimal or mild dysplasia during initial follow-up evaluations were not

Table II. Follow-up after treatment for grade 3 cervical intraepithelial neoplasia

Length of follow-up (mo.)	*No. of patients*	*%*
<6	0	0
6-12	22	4.8
12-24	163	36.1
24-36	114	25.2
36-48	62	13.7
>48	61	13.4
Lost to follow-up	31	7
Total	453	100.0

Table III. Failure rates according to type of freeze

Type of freeze	*No. of patients*	*No. available for follow-up*	*Failures*	
			No.	*%*
Single	42	38	5	13.2*
Double	373	347	13	3.8*
Multiple with or without cautery	38	37	12	32.0

*Difference significant—p = 0.03.

Table IV. Failure rate according to lesion size

Lesion size	*No. evaluable*	*%*	*Failures*	
			No.	*%*
≤1 quadrant	155	37	6	3.9*
>1 quadrant	267	63	24	9*

*Difference significant—p = 0.07.

considered freeze failures for the following reasons: (1) atypical or low-grade dysplastic cells often appear during the first 3 months after cryotherapy as a result of the reparative process, (2) the detection of grade 1 cervical intraepithelial neoplasia was always a transient event, (3) small condylomatous lesions often produced mildly dysplastic cells, (4) no patient needed repeat cryotherapy, and (5) all patients reverted to normal with either no treatment (n = 26, or 60%) or focal cautery (n = 18, or 40%).

Results

During the 11-year period between January, 1972, and December, 1982, 453 patients with grade 3 cervical intraepithelial neoplasia eligible for outpatient treatment were treated with cryotherapy. During this period 31 patients (6.9%) were lost to follow-up, which is defined as less than two Papanicolaou smears within the first year following cryotherapy. The most common reason for losing follow-up on patients was their moving away with no forwarding address to enable further contact. A breakdown of follow-up duration is presented in Table II. Over 50% of patients were followed for ≥2 years. No patient included in the analysis was followed for <6 months. Patients included with follow-up between 6 to 12 months had at least 2 Papanicolaou smears and colposcopic examinations.

Excluding those lost to follow-up (n = 31), the total number of patients available for postcryotherapy analysis was 422. Of these, 392 (93%) were cured with one cryotherapy treatment, while 30 (7.1%) demonstrated persistence and were considered freeze failures (Table III). Forty-four patients (10.5%) were found to have transient stage 1 cervical intraepithelial neoplasia during the first 2 to 12 months following cryotherapy as demonstated by Papanicolaou smear and biopsies in some cases. These patients were not considered as freeze failures for reasons outlined above. Two patients (0.4%) were found to have recurrent disease at 20 and 32 months after cryotherapy and were treated accordingly. Their follow-up has been normal to May, 1984.

The mean age of all 453 patients was 27.6 years (range, 17 to 43 years). The mean age of those with severe dysplasia (313 patients) was 27.9 years and of those with carcinoma in situ (140 patients) was 27.3 years. With respect to age, there was no difference between those who failed treatment (mean = 28 years) and the mean age of the entire group. The vast majority of patients were either nulliparous or nulligravid.

A double freeze was performed on 373 patients and a single freeze on 42. No patients suffered significant complications of cryotherapy. A watery discharge lasting 2 to 3 weeks following treatment was frequently reported, and pads, antibiotic cream, and reassurance were used to alleviate this sometimes bothersome problem.

After double freeze only 13 of 373 patients (3.8%) were found to have persistence, whereas five of 42 patients (13.2%) were found to have persistence after having a single freeze. When lesions were very large (>2 cm) but still confined to the cervix, multiple freezes, with or without peripheral cautery, were often necessary. This form of treatment at one sitting was required by 38 patients. The respective failure rates for these patients are shown in Table III.

The effect of lesion size on the incidence of persistent disease is shown in Table IV. If only one quadrant or less of the cervix was involved with cervical intraepithelial neoplasia, six of 155 patients (3.9%) demonstrated persistence while 24 of 267 patients (9%) with lesions greater than one quadrant demonstrated persistence. Of those patients who were freeze failures (n = 30), only 6 (20%) had small lesions while 24 (80%) had large lesions.

Table V. Results of cryotherapy in grade 3 cervical intraepithelial neoplasia (1972 to 1982)

Histology	*No. of patients*	*No. evaluable*	*No. failed*	*%*
Severe dysplasia	313	289	15	5.2
Carcinoma in situ	140	133	15	11.3
Total	453	422	30	7.1

In analysis of the effect of grade on failure patterns in evaluable patients, 15 of 289 patients with severe dysplasia (5.2%) demonstrated persistence while 15 of 133 patients with carcinoma in situ (11.3%) demonstrated persistence (Table V).

Endocervical gland involvement with stage 3 cervical intraepithelial neoplasia was assessed in 310 patients. After exclusion of those lost to follow-up, 283 patients were left for analysis of the significance of this finding. Thirty-five percent (n = 99) of these patients had gland involvement whereas 65% (n = 184) did not. In the group of those who had persistent disease after cryotherapy, these percentages were identical; 35% of those who failed had gland involvement and 65% did not.

An analysis of the prognostic significance of the factors, lesion size, grade, and type of freeze (single versus double) with respect to persistent disease was carried out for each factor separately (univariate analysis) by means of χ^2– and Fisher's exact test with two-tailed testing. One must be aware, however, of the possible hidden influence of confounding, i.e., the significant effect of one factor on another, when multiple factors of a single disease entity are being assessed individually. In order to investigate and control for the effects of confounding variables, multiple logistic regression analysis was also carried out, again by means of the χ^2– and Fisher's exact test with two-tailed methods. Univariate analysis identified grade ($p = 0.02$) and type of freeze ($p = 0.03$) as significant prognostic factors, while lesion size failed to achieve significance ($p = 0.07$). By the use of multivariate analysis techniques, lesion size remained insignificant ($p = 0.38$), while adjusting for type of freeze and lesion size diminished the apparent significance of grade ($p = 0.33$). Statistical analysis of the difference between the failure rates of double and multiple freezes would be inappropriate as the patients in these groups are not comparable.

There are a number of reasons why one should view these p values with some skepticism. First, despite the fact that lesion size did not prove to be a significant prognostic factor in this analysis, we still observe a higher-than-average failure rate in women with large lesions. Multiple freezes utilizing peripheral cautery or laser is still used to attempt to destroy these lesions at one sitting. Second, statistical analysis and calculations of significance derived from prospective studies that are poorly randomized have to be viewed with caution. The distribution of variables between subgroups is likely to be uneven making comparison of these variables with respect to outcome unreliable. For example, in constructing the table for comparison of freeze technique, lesion size, and grade, we discovered that our randomization process was subject to an unknown bias, since the vast majority (94%) of patients who received a single freeze had severe dysplasia. This weakens the results of the statistical analysis of the comparison of the effect of single versus double freeze on persistence rates with respect to grade of the cervical intraepithelial neoplasia lesion being so treated. It should be noted, however, that other subgroup pairs were fairly comparable in this study.

Of 422 patients, 30 (7%) were found to have persistent disease in the form of grade 2 or 3 cervical intraepithelial neoplasia after cryosurgery. The various methods of re-treatment are shown in Table VI. The disease of 8 patients persisted as grade 2 cervical intraepithelial neoplasia; there was successful re-treatment by focal cautery in three patients, by laser in two patients, and by cone biopsy in one patient. Two patients had spontaneous regression of their persistence. Twenty-two cases persisted as grade 3 cervical intraepithelial neoplasia; 13 were successfully re-treated on an outpatient basis, with cautery in five patients, cryotherapy in five, and laser treatment in three. Surgical treatment was required in only nine patients (2.2%) and consisted of cone biopsy in five of them and total abdominal hysterectomy in the other four. Follow-up on these patients has been normal to May, 1984. When all alternative re-treatment methods are grouped together, 98% of the entire group were successfully treated as outpatients.

One patient demonstrated persistent carcinoma in situ on follow-up biopsy and was found to have microinvasive disease on cone biopsy. She strongly wished to preserve her childbearing function, and subsequent follow-up examinations have been normal to May, 1984. In the entire group no invasive disease was missed.

Comment

During the study it became apparent that grade 3 cervical intraepithelial neoplasia lesion size (Table IV), grade (Table V) and method of freeze (Table III) were the most important variables in predicting freeze failure. Only type of freeze, however, achieved statistical significance. Figge and Creasman[1] noted a 27.5% failure rate with a single freeze in contrast to 8.8% with a double freeze for all grades of cervical intraepithelial neoplasia. With regards to grade 3 cervical intraepithelial neoplasia, this study supports the suggested su-

Table VI. The treatment of freeze failures

Persistence histology	*No. of patients*	*No treatment*	*Cautery*	*Cryosurgery*	*Laser*	*Cone*	*Hysterectomy*
Grade 2 cervical intraepithelial neoplasia	8	2	3	0	2	1	0
Grade 3 cervical intraepithelial neoplasia	22	0	5	6	3	4	4

periority shown in the literature of the double freeze over the single freeze in eradicating disease.[3-7] The double freeze remains the method of choice at our unit. One notes, however, that Benedet et al.[2] treated 347 patients with grade 3 cervical intraepithelial neoplasia with a single 3-minute freeze and found only an 8.9% persistence rate. Such contrasting results make it difficult to determine whether the single freeze is truly inferior to the double freeze or whether operator technique, meticulous patient selection, and overall experience play a more important role in achieving low persistence rates than the actual type of freeze itself. Indeed the operator may have more influence over persistence rates than do the variables—type of freeze, lesion size, grade, and endocervical gland involvement.

Lesion size was noted to be significant by Townsend and Ostergard,[8] DiSaia et al.,[9] Kaufman and Irwin,[10] and Walton et al.[11] Figge and Creasman[1] showed by multiple logistic regression analysis that, when adjustment is made for grade and freezing technique, the apparent significant influence of size disappears for all grades of cervical intraepithelial neoplasia. With regard to grade 3 cervical intraepithelial neoplasia only, lesion size again did not prove to be statistically significant in predicting for persistence after cryotherapy. However, experience indicates that multiple freezes and re-treatment should be anticipated in patients with large lesions. This point needs to be emphasized both to physicians doing cryotherapy and to their patients to ensure that adequate follow-up is maintained and persistence detected. In the present study, 80% of those with persistence had lesions greater than one quadrant in size. Thus, while statistical significance is not strictly demonstrated between the two lesion sizes, there is a significant clinical difference present.

With respect to grade, it is initially difficult to understand why there is so much difference in persistence rates between severe dysplasia (5.1%) and carcinoma in situ (12%) when only two to three cell layers in the upper strata of the neoplastic epithelium differentiate the two. After further analysis this difference looks more apparent than real since the majority of patients with carcinoma in situ lesions (69%) had large lesions. The failure rate in the carcinoma in situ group is thus higher as a result of the confounding effect of size on grade. This observation is consistent with the analysis and statement of Creasman et al.[12] that the "larger lesion is more likely to be found with a higher grade of cervical intraepithelial neoplasia." The distribution of small and large lesion sizes among patients with severe dysplasia in our study was more even (41% versus 59% respectively).

There continues to be concern expressed that dysplasia or carcinoma in situ present deep within endocervical glands is actually outside the 5 mm range of freezing effect, thereby placing the patient at risk for persistence and detection difficulties during follow-up.[13] In this study the incidence of gland involvement in those who failed was equal to that of those who did not fail. This observation does not support the contention that endocervical gland involvement remains untreated and follow-up detection compromised in a significant number of cases.[12] It is likely that untreated cervical intraepithelial neoplasia within the glands or crypts results either from improper probe application and/or nontherapeutic temperatures. Once these technical problems are overcome, endocervical gland involvement should not present a significant problem.

Proving to be significant only if excluded or uncorrected during the necessarily meticulous evaluation and treatment protocol would be other variables, such as (1) less than ideal equipment,[1,3,4] e.g., single probe design or inadequate freezing temperatures due to low tank pressures; (2) improper patient selection,[14] e.g., not using endocervical curettage,[3,10] patient too old,[15] or unrecognized disease in the canal[2]; (3) less than ideal freezing technique, e.g., inability of the probe to destroy areas of dysplasia in endocervical areas or in scarred folds on the cervix[2,7]; difficulty in obtaining a uniform ≥5 mm depth of destruction[16]; inability to obtain a 4 to 5 mm ice ball around the circumference of the probe.[1]

Reference has been made to the pitfalls of statistical analysis when prognostic variables within patient subgroups are compared in a prospective but nonrandomized, nonstratified study. Despite the claims made here and elsewhere[12] with regard to the statistical significance and insignificance of various prognostic factors, their true status in predicting for treatment failure will only be ascertained in a randomized clinical trial.

In such a study, patients would be allocated to the treatment modalities (single versus double freeze) in a controlled randomized fashion. Stratification of prognostic factors, e.g., grade, lesion size, and endocervical gland involvement between the treatment groups would be necessary. In addition, and equally as important, vigorous attempts would have to be made to minimize the strong biases of operator technique and treatment method preferences.

This study shows that at the present time cryotherapy remains an effective, practical, and safe method of treating grade 3 cervical intraepithelial neoplasia. A >90% cure rate with an initial double freeze can be anticipated and an overall 97% cure rate after repeat outpatient treatment for persistence can be achieved. A very rigid method of patient selection and treatment must be employed. Invasive disease must be ruled out. Only the experienced colposcopist and cryotherapist who uses meticulous technique will achieve the success rates noted here. Failure to conform to these methods will result in unacceptable persistence rates. Follow-up must be carried out in an ongoing fashion over an 18- to 24-month period in order to detect persistence and recurrence. Treatment failures should be anticipated, especially in those receiving a single 3-minute freeze or multiple freezes for large lesions and in patients with lesions occupying more than one quadrant of the cervix regardless of the method of freeze. The long-term results are pleasing to both the patient and her gynecologist, especially as recurrence rates are extremely low. Cone biopsy or hysterectomy is avoided in most, and preservation of normal reproductive function is achieved.

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Obstructive uropathy associated with endometriosis

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Although endometriosis involving the urinary tract is uncommon, it is responsible for considerable morbidity. A review of eight cases at two Ottawa teaching hospitals from 1979 to 1983 revealed obstructive uropathy in seven patients. There was permanent loss of kidney function in two, ovarian remnant syndrome in two, and patient and family history of renal disease in three. The diagnosis of endometriosis was not made before operation in four patients. Endometriosis was localized in four patients and generalized in the remaining four, while four patients had significant uterosacral nodularity. The conclusion reached after study of this small but important population is that physicians should have a heightened awareness of this uncommon but serious manifestation of the disease. Earlier diagnosis might be achieved on the basis of a high index of suspicion and careful physical and pelvic examination. The liberal use of intravenous pyelography even in cases of minimal endometriosis is urged. Intensive and prolonged follow-up of all patients with the diagnosis of endometriosis is recommended until the menopause has been reached. Treatment of obstructive uropathy requires meticulous surgical intervention and we recommend ovarian ablation with adjuvant hormonal therapy in most circumstances. (AM J OBSTET GYNECOL 1985;151:207-11.)

Key words: Endometriosis, obstructive uropathy

When a 21-year-old woman presented in chronic renal failure due to undiagnosed ureteral endometriosis, a search for similar cases was initiated. Although endometriosis occurs in 15% to 20% of women during the reproductive years, only 1% of cases involve the urinary tract.[1] While bladder involvement is more common, ureteral endometriosis contributes to significant morbidity from hydroureter to hydronephrosis and eventual loss of renal function. Less than 100 cases of ureteral obstruction due to endometriosis were reported prior to 1979.[2] Because of the paucity of specific symptoms the diagnosis is often not made until significant damage has occurred, and 30% of these patients permanently lose a kidney.[1,3,4] Although recommended treatment is removal of all ovarian tissue and ureteral dissection and/or reimplantation, with excision of endometriotic foci, many physicians are reluctant to extirpate the ovaries before menopause. Danazol and progestins have been used with modest success as primary therapies in uropathy in a few younger patients desirous of childbearing; however, their use is considered suboptimal and experimental at this time.

Material and methods

A retrospective study was done at two Ottawa teaching hospitals from 1973 to 1983. Charts were reviewed and analyzed for age, parity, symptomatology, physical findings, preoperative diagnosis, intravenous pyelogram and retrograde pyelogram results, findings at operation, renal function tests, and management.

Case reports

Case No. 1. This 21-year-old, nulligravid woman was referred from Northern Quebec in renal failure. She had a 5-year history of hypertension, chronic left flank pain, and recent edema, fatigue, and dyspnea. She had been hospitalized at age 3 for oliguria and pyelitis with no apparent sequelae. Oral contraceptives were prescribed from age 12 to age 16 for severe dysmenorrhea and menorrhea but pelvic findings were not recorded. Family history included maternal hypertension and nephrectomy in a cousin.

On examination, she was obese and edematous, and the blood pressure was 200/120 mm Hg. Workup revealed urea, 40 mmol/L, and creatinine, 1100 μmmol/L of sterile urine with proteinuria (2+); hemoglobin was 96 gm/L. Results of cystoscopic examination were normal but bulb ureterograms (after failure to pass retrograde catheters) showed obstruction of the distal ureters at the site of an extrinsic mass in the cul-de-sac. Investigations demonstrated massive dilatation of intrarenal and extrarenal systems of both kidneys with marked atrophy of the cortex. As a lifesaving measure bilateral percutaneous nephrostomies were performed; the 24-hour urinary output was 100 to 150 ml on the left side and 1400 to 1600 ml on the right side. Renal function improved; creatinine decreased to 532 μmol/L and urea to 15 mmol/L. One of us (P. D.) was then consulted because of the possibility of a pelvic malignancy.

On pelvic examination, a firm, fixed, nodular mass

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Table I. Summary of findings in eight cases of endometriosis

	No.
Symptom	
Urinary tract	
Flank pain	1
Hematuria	1
Bladder irritability	1
Other	
Dysmenorrhea Dyspareunia Abdominal pain	6
Physical findings	
Uterosacral ligament nodularity	4
Adnexal mass	2
Laboratory	
Intravenous pyelography, obstruction	8
Retrograde pyelography, below pelvic brim	7
Renal function tests	
Normal	7
Abnormal	1

was palpated in the pouch of Douglas. With the patient under anesthesia, cul-de-sac needle biopsy and laparoscopy were performed. Biopsy revealed fibrotic tissue with foci of endometriosis; endometrial glands were clearly surrounded by a rim of endometrial-type stroma. At laparoscopy both ovaries were obliterated by endometriosis and fibrosis and numerous blue cysts were seen in the cul-de-sac (American Fertility Society Stage III). At laparotomy and after the exploration of both retroperitoneal spaces the fibrotic mass was found to extend to both pelvic sidewalls at the level of the cardinal ligament and to obstruct both ureters totally. A reimplantation was done on the right side and end-to-end ureterocystostomy were performed. Because there was minimal left renal function, no attempt was made to reimplant the left ureter. After operation there was good urine output, usually 800 ml of urine per 8 hours; urea and creatinine levels remained stable, and the nephrostomy catheters were removed uneventfully. Blood pressure was controlled with Lasix and the antihypertensive Visken, and the patient was placed on a regimen of danazol, 200 mg four times a day. Unfortunately the renal function tests gradually deteriorated, and the hypertension became resistant to medical therapy. Repeated ultrasound examinations showed the right kidney to be small and atrophied and there was indication of chronic parenchymal renal disease. On the left, marked hydronephrosis and dilatation of the proximal two thirds of the ureter were found. By May of 1984, creatinine was 1288 μmol/L and urea, 31 mmol/L. The patient had persistent headaches and blood pressure was 180/110 mm Hg. She was admitted to the hospital for initiation of peritoneal dialysis and is now on home maintenance dialysis (16 months after the diagnosis was made).

Case No. 2. This 38-year-old, nulligravid woman with primary infertility, dyspareunia, and dysmenorrhea was diagnosed as having endometriosis (American Fertility Society Stage II) at laparoscopy in 1975. She was treated sequentially with Enovid and danazol over a 7-year period. Persistent symptoms finally led to total abdominal hysterectomy, left salpingo-oophorectomy, and appendectomy in 1983. Intravenous pyelography was not done before operation. At laparotomy, dense fibrous adhesions surrounded the left adnexa, sidewall, and uterus; the right ovary was considered unremarkable and the ureters were not explored. One month after operation the patient complained of right flank and abdominal pain. Urologic investigations confirmed right hydronephrosis and hydroureter at the pelvic inlet. A retrograde pyelogram demonstrated right hydroureter below the sacroiliac joint with accompanying parenchymal renal atrophy. Laparotomy was performed with right ureterolysis providing good resolution of the obstruction and relief of symptoms. Findings were consistent with localized endometriosis. The right ovary was not removed.

Case No. 3. This 47-year-old, gravida 1, para 0 woman presented in July, 1978, with a 5-month history of left flank pain. Workup revealed left ureteral obstruction, severely compromised left renal function, and positive urine cultures. At laparotomy in November, 1978, minimal endometriosis (American Fertility Society Stage I) confirmed by frozen section obstructed the ureter. Reimplantation of the left terminal ureter was performed. After operation there was no left kidney function. After referral to a gynecologist in March, 1979, she underwent definitive total abdominal hysterectomy, bilateral salpingo-oophorectomy, and appendectomy and was placed on a regimen of danazol after operation. A left nephrectomy was done 7 months later because of persistent positive urine cultures and a nonfunctioning kidney.

Case No. 4. This 24-year-old, gravida 1, para 1 woman was found to have large bilateral adnexal masses when she presented with a 3-month history of dull abdominal pain. An intravenous pyelogram revealed 10 to 15 cm pelvic masses with postobstructive atrophy of the right kidney. Findings at laparotomy were extensive pelvic endometriosis (American Fertility Society Stage III) and right ureteral obstruction at the base of the broad ligament. She was treated conservatively with right salpingo-oophorectomy, left ovarian cystectomy, right ureterolysis, and danazol for 1 year after operation and has done well.

Case No. 5. This 46-year-old, gravida 2, para 0 woman presented with abdominal pain, bladder irritability, and a 15 cm pelvic mass in 1979, 6 years after total abdominal hysterectomy, bilateral salpingo-oophorectomy, and appendectomy for symptomatic endometriosis (American Fertility Society Stage I); she had no menopausal symptoms or estrogen replacement. An intravenous pyelogram showed right hydroureter and hydronephrosis. At laparotomy a large endometriotic cyst was exised and left ureterolysis performed. Postoperative recovery was uneventful.

Case No. 6. This 36-year-old, gravida 3, para 2 woman had left ureteral obstruction diagnosed when an intravenous pyelogram was obtained prior to total

Table II. Proposed etiologies of endometriosis

Year	*Author*	*Theory of etiology*
1895	Van Recklinghausen	Wolffian duct rests
1896-1897	Cullen	Müllerian duct rests; migration of endometrium
1896	Iwanoff	Serosal metaplasia
1897	Ries	Lymphatics
1903	Meyer	Surgical transplantation
1921	Sampson	Retrograde menstruation
1936	Navratil	Venous metastasis
1949	Javert	Composite theory: Direct invasion through uterine wall and beyond; transtubal regurgitation and implantation of endometrial tissue; lymphatic or hematogenous metastasis; implantation of endometrial tissue during operation

abdominal hysterectomy for a large leiomyomatous uterus. At laparotomy the left ureter was embedded in dense adnexal adhesions and chocolate cysts, but there was no other evidence of endometriosis (American Fertility Society Stage I). Subtotal hysterectomy, bilateral salpingo-oophorectomy, and transureteroureterostomy were performed. After operation, the intravenous pyelography was normal.

Case No. 7. This 31-year-old, gravida 4, para 2 woman presented with right lower-quadrant pain and hematuria. She had a long history of dysmenorrhea and menorrhagia, and tubal ligation was done 5 years previously. She had a personal history of renal calculi and childhood glomerulonephritis and a family history of glomerulonephritis. An intravenous pyelography showed right hydronephrosis and ureteral obstruction 5 cm from the ureterovesical junction. At laparotomy a band of endometriosis extended from the cervix to the right sidewall, embedding the ureter (American Fertility Society Stage II). Ureterolysis was performed and danazol was given for 8 months before definitive total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. Before operation an intravenous pyelogram was normal, and the patient had no further symptoms.

Case No. 8. This 38-year-old, gravida 3, para 2 woman underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy for endometriosis in 1979. She had childhood pyelonephritis and a family history of renal disease. In November, 1983, she complained of severe abdominal pain and rectal pressure. An intravenous pyelogram was normal but on laparotomy she was found to have a left cystic endometriotic ovarian mass adherent to the ureter (American Fertility Society Stage I). Excision of the mass and left ureterolysis were performed with good postoperative recovery.

Analysis of results

Of eight Caucasian patients aged 21 to 47 (mean age of 35), four were nulliparous and four had one to four pregnancies. Three patients had personal histories of glomerulonephritis, pyelonephritis, and renal stones, respectively; all three reported a family history of renal disease. The preoperative diagnosis was not made in four. Table I describes symptoms, physical findings, and laboratory results.

Treatment was primarily surgical. To relieve ureteral obstruction, five ureterolyses, one reimplantation of terminal ureter, one transureteroureterostomy, and one ureterocystostomy were done. Five patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy, but, unfortunately, the ureteral obstruction occurred following the ovarian remnant syndrome in two of these patients. Conservative operation (resection of endometriomas and relief of ureteric obstruction with preservation of the ovaries) was done in three patients. As danazol now seems to be the drug of choice for the medical treatment of endometriosis, in this series it was used before operation in four patients in preparation for a definitive surgical procedure. Danazol was used for postoperative ovarian suppression in these patients as well. In one patient with extensive pelvic endometriosis, Danazol failed to prevent obstructive uropathy. Two patients suffered permanent loss of renal function; one of these remains in chronic renal failure. Postoperative recovery in seven patients was excellent.

Comment

Patients with ureteral endometriosis appear to be part of a subpopulation with pelvic endometriosis—differing in treatment goals and management. With uncomplicated pelvic endometriosis, the aim is to improve fertility and relieve symptoms through hormonal treatment.

Although the absolute criteria for diagnosis are histologically demonstrable benign endometrial glands surrounded by stroma, in long-standing disease, periureteral fibrosis occurs. In one patient, histologic examination revealed fibrosis only, although at operation the patient was said to have extensive pelvic endometriosis.

Many etiologies have been offered (Table II), but the consensus is that most ureteral endometriosis arises from pelvic foci of endometriosis.[5] Isolated implants are considered to be secondary to dissemination of endometrial tissue through lymphatic or vascular channels.

Delayed diagnosis and conservative management contribute to the morbidity. Although five of eight patients had uterosacral nodularity or adnexal masses, 50% were not diagnosed as having endometriosis before operation. Pelvic findings were not recorded in three of our patients. Klein and Cattolica,[3] in their review of 62 patients, reported that pelvic examinations were not performed or results were not recorded in 37%. Time to diagnosis averaged 23 months.[3] They recommended reconfirmation of uterosacral nodularity in early menses when implants are larger and more tender. Many physicians do not perform a rectovaginal examination as an adjunct to bimanual pelvic examination; thus evaluation is not complete.

Obstructive uropathy can occur in both minimal and extensive endometriosis.[4, 6] Four of our patients had solitary periureteral endometriomas (American Fertility Society Stage I) while four had more extensive disease (American Fertility Society Stage II in two and Stage III in two).

Intravenous pyelograms and retrograde pyelograms confirmed obstructive uropathy in seven patients, all below the pelvic brim, as consistently reported.[4, 7] Intravenous pyelography is invaluable in demonstrating severity of obstruction, approximate location, and laterality. In addition it provides a baseline for future study. Retrograde pyelography provides precise location, extent of lesion, and the degree of ureteric stenosis. Radiography is not helpful in differentiating the more common extrinsic ureteral endometriosis (external to adventitia) from intrinsic disease (actual invasion of the ureteral wall); both can lead to fibrosis and are not easily differentiated clinically, although cyclic pain and hematuria often occur with intrinsic involvement.[8, 9]

Optimal treatment involves relief of obstruction, resection of endometriotic tissue, and castration. Retroperitoneal exploration is recommended to ensure complete removal of ovarian tissue and to establish the presence and extent of ureteral involvement.[10] Castration alone usually is not helpful since fibrosis causes the ureteral obstruction to remain.[5] When the ovaries are preserved, 27% of patients require further operation while less than 3% of patients treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy require additional surgical procedures for endometriosis (S. J. Behrman, M.D., unpublished data). Although Langmade[10] and Gantt et al.[11] stated that estrogen replacement usually does not cause reactivation of endometriosis, it is our impression that it is a factor in recurrence. However, when endometriosis recurs during estrogen replacement, it can also be due to incomplete removal of ovarian tissue.[10, 12] Dick et al.,[13] in 1973, reported a case of postmenopausal ureteral endometriosis after 11 years of Premarin, 1.25 mg daily.

In our series, obstructive uropathy occurred after conservative operation or danazol or progestin treatment and in the presence of residual ovarian tissue after total abdominal hysterectomy and salpingo-oophorectomy.

When the obstruction is recent, when the patient wishes further childbearing, or when she refuses operation, conservative management with progestins or danazol may be useful.[3, 14] Depo-Provera has successfully relieved obstructive uropathy without operation,[11] while progestin before operation can decrease the size of implants.[15] Although the estrogen-progestin combination pill can initially stimulate endometrial tissue, Lavalle et al.[11] has reported one case where norgestrel–ethinyl estradiol achieved complete resolution of the obstruction. Danazol has been used with good results in several patients.[2, 16] Strict criteria should be used: established diagnosis of endometriosis, informed consent regarding risks, serial intravenous pyelography, careful assessment of overall renal function, state of the unaffected kidney, and patient compliance.[16, 17]

The significance of previous personal and family histories of renal disease is not clear. It is possibly a risk factor.

In order to manage cases of ureteral obstruction we recommend the following steps in diagnosis and treatment. Diagnosis should include: (1) high index of suspicion for persistent symptoms referrable to the urinary tract and the abdomen, dysmenorrhea, unexplained hypertension, and renal abnormality; (2) careful physical examination, especially nodularity of the uterosacral ligaments; (3) routine use of intravenous pyelography once the diagnosis of endometriosis is made, even in minimal cases, and intravenous pyelography before any planned operation for endometriosis; (4) ascertainment of possible risk factors in personal or family history of renal disease; (5) use of the American Fertility Society classification for accurate staging to compare results, to allow comparability between institutions, and to make possible multicenter trials. Treatment should consist of: (1) definitive operation (total abdominal hysterectomy, bilateral salpingo-oophorectomy) in most cases; (2) meticulous surgical technique to ensure complete removal of all ovarian tissue, and (3) exposure and examination of ureters whenever operation for endometriosis is carried out.

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A case report of primary adenocarcinoma of the fallopian tube

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The apparently smallest primary adenocarcinoma (2.3 mm in diameter) of the fallopian tube ever described was diagnosed by repeated blind sectioning of the surgical specimen after the preoperative finding of an abnormal cervical Papanicolaou smear and the subsequent finding of abnormal cells in fluid obtained by laparoscopic peritoneal lavage, although a survey of the patient for a primary cancer was negative. (AM J OBSTET GYNECOL 1985; 151:211-2.)

Key words: Fallopian tube, cancer, microscopic primary adenocarcinoma

This is a case report of the apparently smallest fallopian tube malignancy recorded in the literature to the present.

Case presentation

Mrs. M. M., a 51-year-old gravida 5, para 4, abortion 1, living children 4, postmenopausal schoolteacher, was referred by her family physician because of an abnormal Papanicolaou smear described as "highly suggestive of malignant cells" on her annual checkup on May 15, 1979. The only symptoms the patient described were fatigue and headaches. She had been menopausal for 8 years and she denied postmenopausal bleeding. On examination, she was a thin, healthy-looking woman. A speculum examination showed an atrophic cervix with an endocervical polyp. There was no palpable pelvic lesion.

Investigations, including a repeat Papanicolaou smear, fractional dilatation and curettage, sigmoidoscopy, barium enema, upper gastrointestinal series, gastroscopy, E.R.C.P., liver and spleen scan, chest x-ray film, intravenous pyelogram, computed tomographic scan of the abdomen, and mammography, were all normal.

Laparoscopic examination of the pelvis and abdominal cavity was normal; however, peritoneal lavage with saline at the time of laparoscopy was positive for adenocarcinoma cells. These cells were judged as being compatible with the Papanicolaou smear cells. At laparotomy, no abnormality was detected in the abdominal cavity.

In view of these findings, the suspicion of fallopian tube carcinoma led us to perform a total abdominal hysterectomy with bilateral salpingo-oophorectomy.

The initial pathologic reports described only atrophic changes within the ovaries, tubes, and uterus. A third reexamination of the surgical specimen demonstrated a 2.3 mm focus of adenocarcinoma in the fimbrial end of the right fallopian tube. The oncology pathologist believed that this was a primary tubal carcinoma. Because of the poor differentiation of cells, full-dose pelvic irradiation and abdominal strip radiation were carried

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out. The patient was well until 18 months later when she returned with intestinal obstruction. At laparotomy, carcinoma metastatic to the liver, intestines, and mesentery was identified, but no primary site was found. Biopsy showed poorly differentiated adenocarcinoma, most likely secondary, compatible with previous findings in the fallopian tube. The patient died within 24 months of the secondary diagnosis. The family refused autopsy.

Comment

The incidence of fallopian tube carcinoma varies from 0.1% to 0.5% in the age group of 18 to 80 years.

Early diagnosis of fallopian tube cancer is a difficult problem. Diligent investigations and persistence on the part of the physician are important. The emphasis is placed on laparoscopic peritoneal lavage in the presence of positive cervical cytologic findings and negative curettage for diagnosis of fallopian tube carcinoma.

Fiedler and Lock[1] have demonstrated the value of the cervical smear as an aid in diagnosis of carcinoma of the fallopian tube.

Sedlis[2] reported the presence of tubal tuberculosis coexistent with tubal malignancy. A finding of note was the apparently high percentage of nulligravid patients.

Hanton et al.[3] from the Mayo Clinic reported 27 patients with primary carcinoma in the fallopian tube. During the 20-year period, the treatment of choice was total abdominal hysterectomy and bilateral salpingo-oophorectomy.

Boutselis and Thompson[4] reporting 14 cases between 1950 and 1970 of primary carcinoma of the fallopian tube agreed with previous reports that the optimum treatment was total abdominal hysterectomy and bilateral salpingo-oophorectomy. When the tumor has spread into the pelvis, postoperative irradiation is indicated. A more radical pelvic operation is indicated in selected cases and chemotherapy may be a valuable adjuvant.

The most important prognostic factor is the clinical stage of the disease when therapy is administered.

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The changing concepts of condyloma

A retrospective study of colposcopically directed cervical biopsies

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Human papillomavirus has been implicated in the etiology of cervical intraepithelial neoplasia. A retrospective audit was conducted on all colposcopically directed biopsies performed from June through November, inclusive, in the years 1980 and 1982, a total of 317 cases. In 1980, the misdiagnosis rate for condyloma was 88.9% (69 of 78) and, in 1982, the misdiagnosis rate was reduced to 28.5% (40 of 140). On review, the association of cervical intraepithelial neoplasia with condyloma was 73% (107 of 146), $p < 0.005$. The differences in mean ages of patients with condyloma alone, cervical intraepithelial neoplasia with condyloma, and cervical intraepithelial neoplasia alone were significant, $p < 0.02$. When condyloma is not recognized, the pathologist tends to overdiagnose grade 3 cervical intraepithelial neoplasia as shown in 28 original cases; 16 of 28 (57.1%) were condyloma alone on review. Fifteen (53.6%) of these 28 patients underwent a cone biopsy or hysterectomy when a less radical procedure would have been acceptable. The morphologic changes indicating cervical condyloma were not fully appreciated by the pathologist, and this resulted in overdiagnosis and later overtreatment by the gynecologist. (AM J OBSTET GYNECOL 1985;151:213-9.)

Key words: Condyloma, human papillomavirus, cervical intraepithelial neoplasia, uterine cervix

Condyloma acuminatum or genital warts of the vulva, perineum, anus, vagina, and occasionally the cervix are well-described lesions associated with sexual transmission.

Koss and Durfee,[1] in 1956, defined koilocytotic atypia of the uterine cervix. In 1976 and 1977, Meisels et al.,[2,3] described the appearance of flat condyloma (condyloma planum) by cytologic, colposcopic, and histopathologic characteristics. They suggested that condyloma may be involved in the initial developmental steps of cervical intraepithelial neoplasia. As early as 1970, Oriel and Almeida[4] demonstrated the presence of intranuclear human papillomavirus particles in genital warts, and human papillomavirus particles were identified, in 1981, by Casas-Cordero et al.[5] within koilocytes of cervical condyloma by means of transmission electron microscopy. Immunoperoxidase staining confirmed the electron microscopic studies, indicating the presence of human papillomavirus antigen in the nuclei of koilocytes.[6]

In 1981, Meisels et al.[7] stated that atypical condyloma, characterized by nuclear atypia with polyploid DNA values, mimics cervical intraepithelial neoplasia. Of 110 patients with atypical condylomas followed up for 1 year in their study, 100 cases reverted to normal on Papanicolaou smear while 10 cases progressed to grade 3 cervical intraepithelial neoplasia (severe dysplasia and carcinoma in situ), suggesting a close association between human papillomavirus infection and carcinoma of the cervix. Boon and Fox,[8] in 1981, described the simultaneous appearance of condyloma and cervical intraepithelial neoplasia, and, in 1982, Fenoglio and Ferenczy[9] reiterated the close association between condyloma and cervical intraepithelial neoplasia. Furthermore, Crum, et al.,[10] in 1983, found the mean age of women with condyloma alone was less than that of women with cervical intraepithelial neoplasia and condyloma. The group with cervical intraepithelial neoplasia alone was the elder.

The classical condyloma acuminatum of the genital area is an exophytic lesion well known by both pathologists and gynecologists. The confusion among cytologists, colposcopists, and histopathologists has arisen with the flat, the atypical, and the endophytic types of condyloma. Recent correlative morphologic,[11] Feulgen

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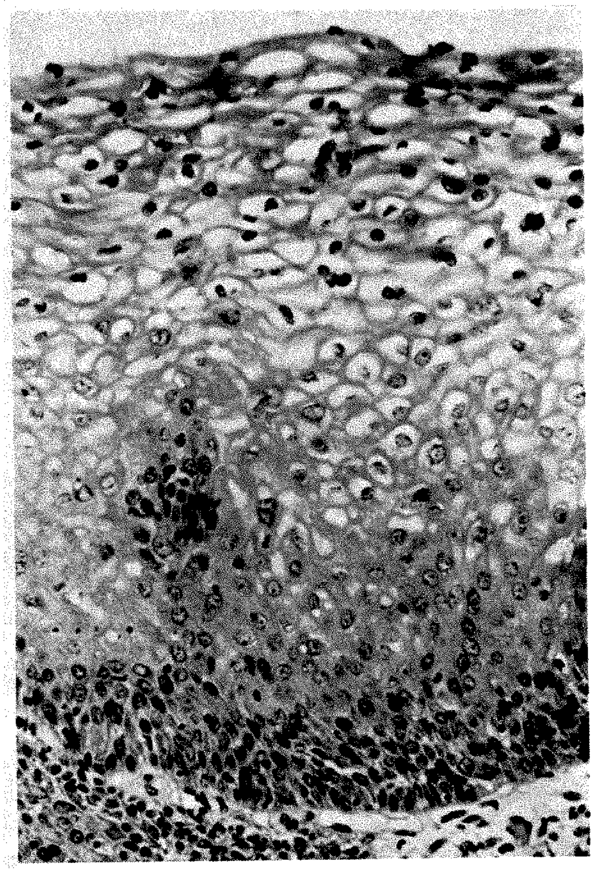

Fig. 1. Flat condyloma (planum) of cervix. Koilocytotic cells with cytoplasmic ballooning and enlarged degenerative and often double nuclei occupy the upper one third of the epithelium. The basal and parabasal layers contain normal to slightly reactive cells. (× 100.)

DNA microspectrophotometric quantitation,[12] molecular hybridization,[13-16] and clinical studies identified two distinct types of cervical condylomatous lesions.[11, 12] The first is characterized by nuclear polyploidy, lack of mitotic figures other than tripolar mitosis and dispersed metaphases,[17] and human papillomavirus DNA types 6 and/or 11.[13, 14, 16] Such lesions are ordinary condylomas, tend to regress spontaneously or not to recur after treatment, and have virtually no known malignant potential. The second type is associated with nuclear aneuploidy and abnormal mitotic figures and contains human papillomavirus DNA types 16[15, 16] and/or 18.[16] This type of lesion is designated as cervical intraepithelial neoplasia with koilocytotic features; it tends to persist, rarely regresses, and apparently has a relatively high malignant potential.

The purpose of this study was to determine the extent to which the "condyloma concept" had penetrated the pathologic practice in our institution. Consequently, we attempted to identify: (1) any change in the histologic recognition and diagnoses of condyloma, (2) the rate of association between condyloma and cervical intraepithelial neoplasia, and (3) the treatments received when condyloma was originally overdiagnosed as grade 3 cervical intraepithelial neoplasia.

Material and methods

All colposcopically directed biopsies from the periods June to November, inclusive, 1980 and 1982, were reviewed by a resident and a senior pathologist. The review took place in October through December, 1982. A second senior pathologist and a gynecologic pathologist reviewed all cases that were originally diagnosed as grade 3 cervical intraepithelial neoplasia. There were 317 colposcopically directed biopsy specimens that had been fixed in formalin and embedded in paraffin. Originally each block was cut into eight sections with two or three subsections of 5 to 6 μm in thickness. Cuts numbered 2, 4, 6, and 8 were stained with hematoxylin and eosin.

The criteria used for the diagnosis of ordinary condyloma of the cervix were as published by Meisels and Fortin.[2] The thickness of the squamous epithelium may be variable. Basal and parabasal layers have normal cellular polarization. The upper one third to two thirds of the epithelium contains koilocytes (Fig. 1). The surface layers often have cells featuring intracellular keratinization or dyskeratotic changes. Nuclei of the deeper cell layers are relatively large, as are the nucleoli, but the chromatin is finely dispersed. These alterations are consistent with nuclear polyploidy, that is, reactive or hypertrophic changes. The nuclei of the upper cell layers have rather a large and retracted, hyperchromatic, sometimes pyknotic or karyorrhectic, appearance. These changes are a reflection of degeneration. There may be binucleation or multinucleation. The koilocyte is a large cell with a relatively large, hyperchromatic nucleus surrounded by an irregularly shaped cytoplasmic cavity (cytoplasmic ballooning). The chromatin is often agglutinated with peripheral margination suggestive of degeneration. Abnormal mitotic figures are absent.

Occasionally condyloma extends into the subjacent endocervical "glands," replacing and obliterating the columnar epithelium. In other instances, the condylomatous changes occur in squamous metaplastic epithelium which has already replaced endocervical "glands." In either situation the term endophytic or inverted condyloma is used.

Condyloma and cervical intraepithelial neoplasia may be seen separately in the same specimen, or condyloma may be interspersed within areas of cervical intraepithelial neoplasia[8] or be present in the upper levels of the abnormal epithelium (Figs. 2 and 3). In the last group, the term "cervical intraepithelial neoplasia with condylomatous or koilocytotic features" is used. Regardless of grade, cervical intraepithelial neo-

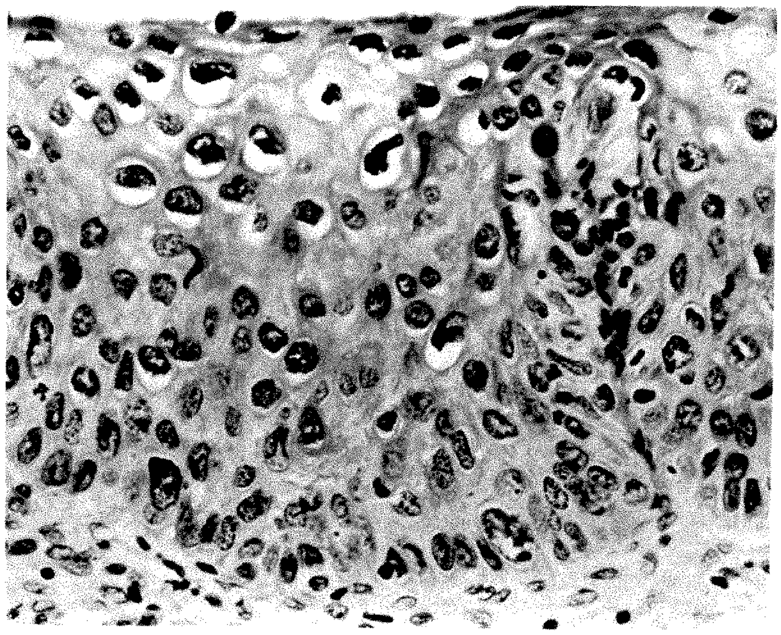

Fig. 2. Cervical intraepithelial neoplasia, grade 1 (mild dysplasia), with koilocytotic features. The lower one third of the epithelium contains atypical, pleomorphic nuclei displaying loss of cohesion and organization and coarse, often aggregated chromatin. Such cells may have aneuploid nuclear DNA content. The upper two thirds to one half of the epithelium has koilocytotic cells, many of which contain nuclear degenerative changes. (Original magnification ×250.)

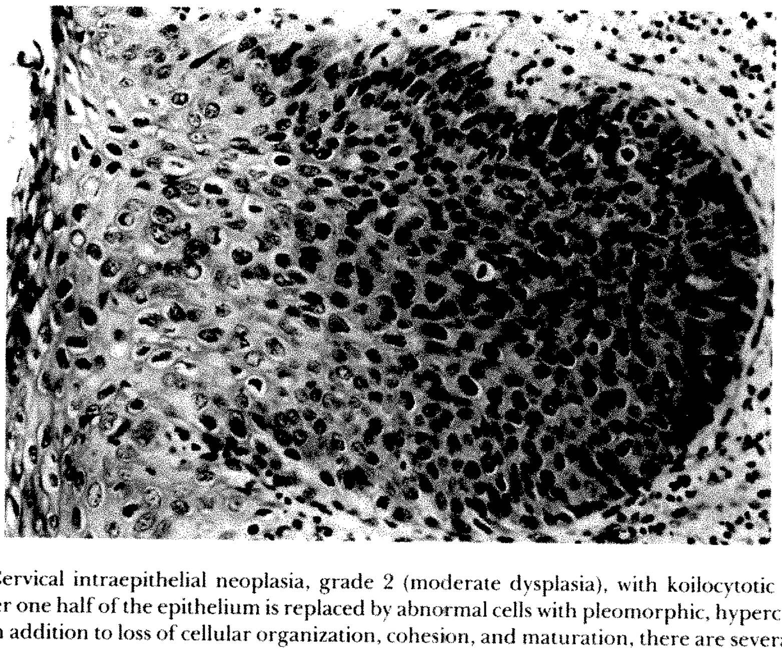

Fig. 3. Cervical intraepithelial neoplasia, grade 2 (moderate dysplasia), with koilocytotic features. The lower one half of the epithelium is replaced by abnormal cells with pleomorphic, hyperchromatic nuclei. In addition to loss of cellular organization, cohesion, and maturation, there are several mitotic figures. The upper one half of the epithelium demonstrates an attempt at cytoplasmic maturation, although the nuclear morphologic features remain abnormal. The most superficial cells have irregular cytoplasmic cavities and dark, degenerative nuclei consistent with koilocytotic atypia associated with presence of human papillomavirus. (Original magnification × 150.)

plasia has abnormal cells in which the nuclei have aneuploid DNA content and abnormal mitotic figures. However, where there is cervical intraepithelial neoplasia, the basal layer is always involved with abnormal cells. The cells in the upper epithelial strata demonstrate koilocytotic atypia. The so-called atypical condyloma has all the cytologic features of cervical intraepithelial neoplasia.

The term unclassifiable cervical intraepithelial neoplasia has been used for those lesions in which accurate

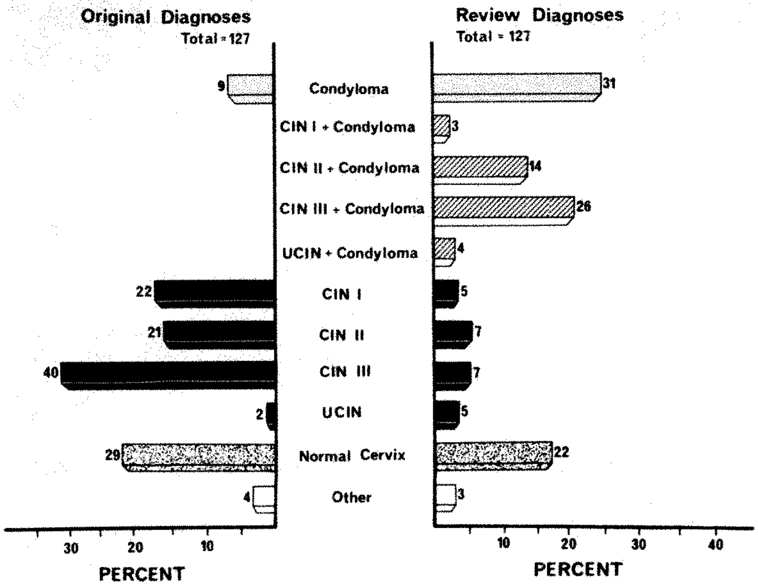

Fig. 4. Histopathologic results of colposcopically directed biopsies, June through November, 1980, inclusive. (*CIN* = Cervical intraepithelial neoplasia and *UCIN* = unclassifiable *CIN*.)

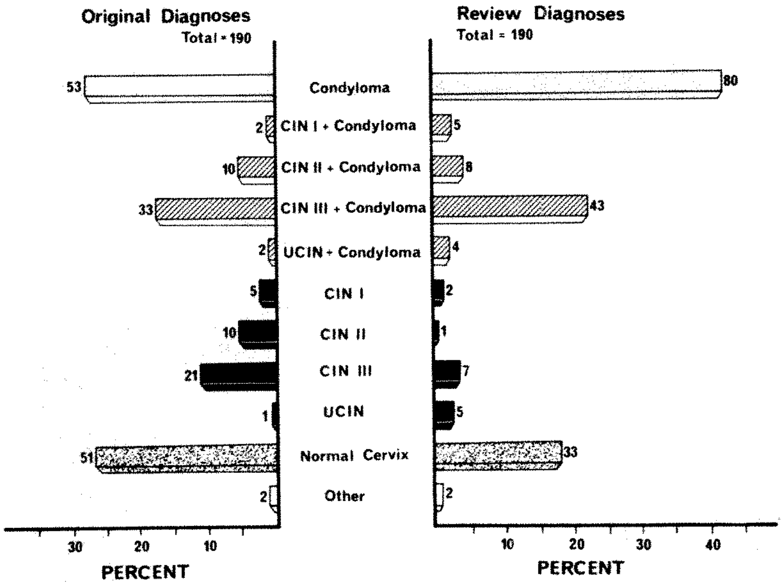

Fig. 5. Histopathologic results of colposcopically directed biopsies, June through November, 1982, inclusive. (*CIN* = Cervical intraepithelial neoplasia and *UCIN* = unclassifiable *CIN*.)

grading could not be provided because of the lack of full thickness of surface epithelium. However, the epithelial cells that are visualized demonstrate unequivocal intraepithelial neoplastic features.

To compare the association of cervical intraepithelial neoplasia with condyloma to condyloma alone the χ^2 analysis was used. The Z test was used to compare the proportion of patients who were aged ≤ 35 against those who were older than age 35. An analysis of mean ranks was conducted with the Kruskal-Wallis test, as well as an analysis of variance with the use of Scheffe's F test. The Mann-Whitney test was used to compare the significance of median ages.

Results

The original diagnosis of 127 colposcopically directed biopsies between June and November, inclusive, of 1980, 4 years after the original article of Meisels and Fortin,[2] revealed only nine of 127 (7%) cases of condyloma alone and none of cervical intraepithelial neoplasia with condyloma (Fig. 4). The review of these biopsies revealed 31 (24.4%) cases of condyloma alone and 47 (37%) cases of cervical intraepithelial neoplasia with condyloma. Of the total cases 78 of 127 (61.4%) finally diagnosed as condyloma, 69 of 78 (88.5%) were initially missed.

Two years later, from June to November, 1982, inclusive, there were 190 colposcopically directed biopsies (Fig. 5). The original diagnoses were condyloma alone in 53 (27.9%) and cervical intraepithelial neoplasia with condyloma in 47 (24.7%). On review there were 80 of 190 (42.1%) cases of condyloma alone and 60 (31.6%) of cervical intraepithelial neoplasia with condyloma. A total of 40 of 140 (28.5%) cases of condyloma were initially missed.

With both review periods combined, there were 111 cases of condyloma alone. Of these, five of 111 (4.5%) were exophytic, four (3.6%) were endophytic, and 102 (91.9%) were flat condylomas. On review there were 146 of 317 (46%) cases with cervical intraepithelial neoplasia; of these, 107 of 146 (73%) cases were neoplasia with condyloma. The association of cervical intraepithelial neoplasia with condyloma when compared to cervical intraepithelial neoplasia alone is highly significant (χ^2 analysis with 4 df = 16.07, $p < 0.005$, Fig. 6). The incidence of grade 3 cervical intraepithelial neoplasia with condyloma as compared to neoplasia alone was also highly significant (χ^2 analysis with 1 df = 10.80, $p < 0.01$, Fig. 6).

From the combined periods of 1980 and 1982, 66 of the original 94 cases diagnosed as grade 3 neoplasia were confirmed, 11 as neoplasia alone and 55 as neoplasia with condyloma. However, 28 (29.8%) of the original 94 cases were reclassified as follows: 16, condyloma alone; two, grade 1 neoplasia with condyloma; four, grade 2 neoplasia with condyloma; four, unclassifiable neoplasia with condyloma; and two, unclassifiable neoplasia alone. Twenty-six (92.9%) cases were associated with condyloma. Of the 17 new cases of grade 3 cervical intraepithelial neoplasia diagnosed, 11 were originally called condyloma, three were grade 2, one was microinvasive carcinoma, and two were unclassifiable neoplasia.

Analysis of the age distribution (Fig. 7) revealed 194 of 218 (89%) patients with condyloma alone and neoplasia with condyloma were aged ≤35 as compared to 24 (11%) patients who were older than age 35 (Z test, $p < 0.00001$). When the mean ranks were analyzed with the Kruskal-Wallis test, the differences in these mean ranks were significant (H value = 6.36, $p < 0.05$), between the groups with condyloma alone, neoplasia with condyloma, and neoplasia alone (Table I). An analysis of mean ages using the analysis of variance also shows the mean to be significantly different (F value = 4.28, $p < 0.02$, Table I). An analysis of the median ages showed the group with condyloma alone to have a median age of 27 years, which is significantly younger than the group with neoplasia alone, that had a median age of 31 years ($p < 0.03$, Mann-Whitney test, Table I). The age of peak frequency in the group with condyloma alone was 24, while that in the group with neoplasia alone was 30 (Table I).

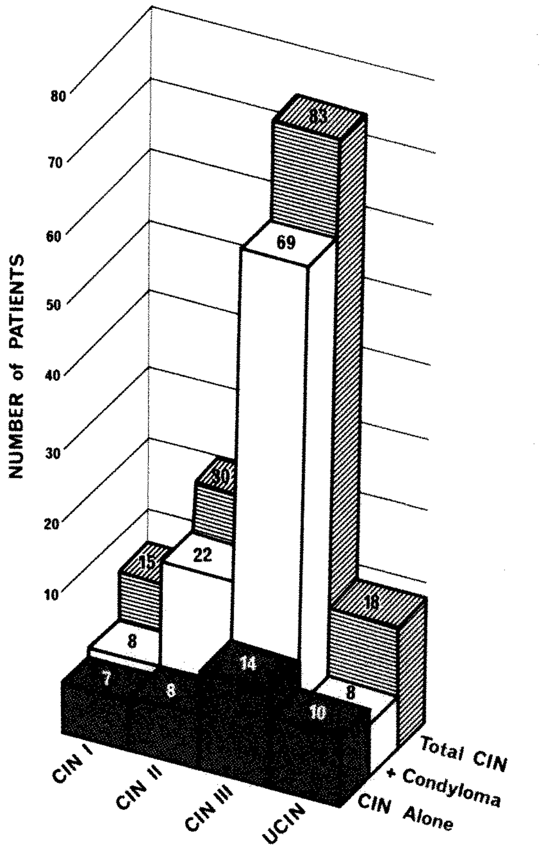

Fig. 6. Review diagnoses of condyloma associated with cervical intraepithelial neoplasia, June through November, inclusive, in the years 1980 and 1982. The incidence of cervical intraepithelial neoplasia associated with condyloma (107 of 146 or 73%) is highly significant when compared to that of neoplasia alone (39 of 146 or 26.7%, $p < 0.005$). The incidence of grade 3 cervical intraepithelial neoplasia with condyloma (69 of 83 or 83.1%) is highly significant when compared with that of grade 3 neoplasia alone (14 of 83 or 16.9%, $p < 0.01$). (*CIN* = Cervical intraepithelial neoplasia and *UCIN* = unclassifiable *CIN*.)

Comment

The cytologic, colposcopic, and histopathologic features of condyloma heralded by the publications of Meisels et al.,[2, 3] in 1976 and 1977, did not lead to instant and universal recognition of this viral infection. In the years that followed, little was published regarding colposcopic appearance and clinical implications. It is understandable, therefore, that clinicians and pathologists were confused, and many looked at the condyloma concept with skepticism.

This study demonstrates that new information about

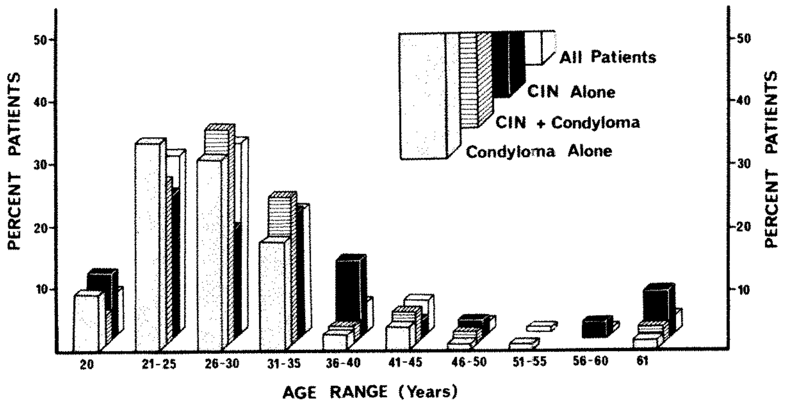

Fig. 7. Age distribution of all the patients with colposcopically directed biopsies, June through November, inclusive, in the years 1980 and 1982. A total of 194 of 218 (89%) patients with condyloma alone and cervical intraepithelial neoplasia with condyloma were ≤35 years old as compared to 24 (11%) patients who were >35 years old (Z test, $p < 0.00001$).

Table I. Age distribution analysis

	Condyloma alone	*Cervical intraepithelial neoplasia with condyloma*	*Cervical intraepithelial neoplasia alone*	*Significance*
Total patients	111	107	39	
Mean age (±SD)	28.35 ± 8.09	29.80 ± 9.07	33.54 ± 13.74	$p < 0.02$*
Mean rank	117.42	133.26	150.26	$p < 0.05$†
Median age	27‡	28	31‡	
Age at peak frequency	24	29	30	

*Analysis of variance.

†Kruskal-Wallis test.

‡Mann-Whitney test (statistically significant difference between the condyloma alone and cervical intraepithelial neoplasia alone groups only, $p < 0.03$).

condyloma improved the diagnosis between the 1980 and 1982 study periods. Of the cases of condyloma alone, 106 of 111 (95.5%) were of the flat or endophytic type. These 106 cases appeared as a subclinical infection, not recognized by gross inspection or seen with difficulty on colposcopy, mainly as an acetowhite patch. The exophytic condyloma, which can be seen on gross inspection and is familiar to all gynecologists, accounted for 4.5% of the group with condyloma alone. This distribution of the types of cervical condyloma may be a contributing factor to the lack of earlier acceptance of the "condyloma concept."

The incidence of cervical intraepithelial neoplasia associated with condyloma, 107 of 146 (73.3%), is highly significant when compared to that of neoplasia alone, 39 of 146 (26.7%) ($p < 0.005$). The incidence of grade 3 neoplasia with condyloma, 69 of 83 (83.1%), when compared with that of grade 3 neoplasia alone, 14 of 83 (16.9%), shows a highly significant difference, $p < 0.01$. However, there was no such significant difference in our study with grades 1 and 2 cervical intraepithelial neoplasia with or without condyloma. Colposcopy was initiated in 1976 in our referral area, and by 1980, there were two colposcopists for a population of approximately 500,000. When the Papanicolaou smears prior to the colposcopically directed biopsies were analyzed, 118 of 238 (49.6%) patients had abnormal smears for a period ranging from 1 to 8 years before their first colposcopic investigation. This situation prevailed for 69% of patients within the grade 3 neoplasia with condyloma group which explains the disproportionate number of cases of grade 3 relative to grades 1 and 2 (Figs. 4 and 5).

Epidemiologically, we have confirmed, together with others,[10] that the group with condyloma alone (age 28.35 years) was younger than the group with neoplasia and condyloma (age 29.8 years). This suggests that condyloma may be a precursor of cervical intraepithelial neoplasia associated with condyloma. Of all patients with condyloma, condyloma alone, and cervical intraepithelial neoplasia with condyloma, 89% were aged ≤35, $p < 0.00001$.

Originally there were 94 cases of grade 3 cervical intraepithelial neoplasia with or without condyloma.

On review there were only 66 cases, which represents a net overdiagnosis rate of 29.8%. This was due to the presence of condyloma. In the original group of grade 3 neoplasia, there were 28 cases on review which were not grade 3. Sixteen of 28 (57.1%) cases were condyloma. The presence of condyloma may lead to an overgrading of cervical intraepithelial neoplasia by pathologists who are unfamiliar with the diagnosis of condyloma. Such overreading may have clinical implications to the practicing gynecologist regarding the selection of therapy. Indeed, 15 condylomas that were originally diagnosed as grade 3 neoplasia would have been less radically treated with cryotherapy or laser therapy. Instead 11 patients underwent cone biopsy and four patients underwent hysterectomy. These 15 patients had an unnecessary and costly procedure with an increased potential risk of morbidity and mortality. The psychological aspect of having grade 3 cervical intraepithelial neoplasia diagnosed is traumatic to the patient. Of the 17 new grade 3 cases diagnosed on review, there were originally 11 in the group with condyloma alone, indicating a problem with underdiagnosis as well. These 17 patients received adequate treatment, including cryotherapy in 11, conization in five, and hysterectomy in one.

This study highlights the importance of providing an accurate histologic diagnosis of cervical biopsies and understanding the natural history, including morphologic manifestations of the human papillomavirus infection–cervical intraepithelial neoplasia complex. If human papillomavirus infection of the cervix is not recognized, the lesion may be overdiagnosed as cervical intraepithelial neoplasia, leading to overtreatment. On the other hand, an initial diagnosis of grade 2 or 3 cervical intraepithelial neoplasia may on review be reclassified as a lower grade of neoplasia with condyloma, again leading to overtreatment. Also, failure to understand the appearance of condyloma may occasionally lead to an underdiagnosis. The association of condyloma with cervical intraepithelial neoplasia is highly significant, $p < 0.005$. Reid et al.[18] also found a significant association between condyloma and cervical intraepithelial neoplasia as well as between condyloma and invasive cervical carcinoma. In fact, it has recently been suggested that human papillomavirus of DNA types 16[15, 16] and 18[16] may be related etiologically to cervical cancer precursors and invasive cancer. Consequently, a cytologic diagnosis of condyloma must be followed up with a colposcopic evaluation of the patient to rule out coexistent cervical intraepithelial neoplasia. Pathologists and gynecologists who are familiar with the condyloma issue are likely to provide the best possible care to their patients.

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The epidemiology of urethral-vesical dysfunction in the female patient

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The specific purposes of this report are twofold: (1) to summarize the descriptive epidemiology of urethral-vesical dysfunction in a population of 1439 female patients referred for urodynamic evaluation of symptoms and (2) to analyze the association between demographic, clinical, and investigative data. On the basis of urodynamic studies, abnormalities were classified into four types: anatomic dysfunction, 60%; neuromuscular dysfunction, 20%; neuropathic dysfunction, 12%; and miscellaneous dysfunction, 8%. Analyses and cross tabulations of clinical data *vis-à-vis* investigative studies reveal poor correlation between subjective clinical data and urodynamic diagnoses. The clinical symptoms of urethral-vesical dysfunction in the female patient may be confusing and complex. Urodynamic evaluation of symptoms and clinical data is of value in the design of a rational therapeutic program for such patients. (Am J Obstet Gynecol 1985;151:220-3.)

Key words: Epidemiology, dysfunction, urethral-vesical

The problem of lower urinary tract dysfunction is well known to every category of clinical physician, and these patients are to be found throughout the total spectrum of clinical practice. There have been very few epidemiologic studies of the problem and the majority of these have been confined to the geriatric patient. However, Thomas et al.[1] studied a population sample of 22,430 patients who were more than 5 years old in whom there was a prevalence of lower urinary tract dysfunction of 9.2% among male subjects and 7.8% among female subjects. Yarnell et al.[2] reported upon 1060 randomly selected women older than age 18, 45% of whom admitted to some degree of incontinence and 3% of whom reported symptoms of a sufficient degree to interfere with their social or domestic life. In addition to the significant prevalence of this syndrome of urethral-vesical dysfunction in the female patient, there is the problem of appropriate therapy when such therapy is based upon subjective symptoms and clinical findings. Jeffcoate and Francis[3] wrote of the difficulties of determining the type of incontinence from which a woman suffers; Bates et al.[4] described the urinary bladder as often proving to be an "unreliable witness." Farrar et al.[5] stated that of women who complain of stress incontinence as a pure and solitary symptom, 90% will have anatomic sphincteric insufficiency of the urethral-vesical neck, but of women who complain of stress and urgency incontinence, 80% of these patients will, upon study, be found to have detrusor instability. Powell et al.[6] reported upon the study of 1800 women with symptoms of urethral-vesical dysfunction, independently classified by two different experts as to type of incontinence, one investigator basing his diagnosis upon a detailed clinical history and examination and the other investigator, upon urodynamic investigation only. When the diagnoses of the two investigators were compared, the correlation of diagnoses by the two individuals was poor. Of the patients clinically diagnosed as having stress incontinence, the urodynamic studies provided substantive evidence of a diagnosis in only 50% of patients. The clinical diagnosis of urgency incontinence was urodynamically confirmed in 30% of patients, and 10% of the urgency incontinence group, by clinical diagnosis, were identified, following urodynamic evaluation, as having genuine stress incontinence. The conclusion of those studies was that it is extremely difficult for an experienced investigator to predict the urodynamic findings in certain clinical groups on the basis of patient symptoms and the clinical examination alone.

Methods and material*

A total of 1439 of the patients referred to our unit for investigation and diagnosis were evaluated by a detailed gynecologic-urologic clinical history and ex-

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*Methods, definitions, and units conform to the standards proposed by the International Continence Society, except when specifically noted.

Table I. Referral source of study population (1439 patients)

Source	%
Physician	
Family physician	65
Gynecologist	16
Other consultants	19
Region	
Metropolitan area	68
Niagara health region	32

Table II. Urodynamic classification (1439 patients)

Classification	%
Anatomic incontinence	60
Idiopathic neuromuscular incontinence	20
Neurological incontinence	12
Mixed-type incontinence	8

amination and underwent a comprehensive urodynamic study. Clinical and investigative data were recorded for storage, retrieval, and follow-up purposes. The time span of these studies was 5 years (1977 through 1981).

Urodynamic investigational-diagnostic procedures consisted of: uroflow studies; cystometrograms obtained with medium-flow vesical filling, normal saline at body temperature being the infusion medium of first choice; urethral-vesical pressure profilometry with a four-channel Fr. 10 catheter; sphincter electromyography; cystoscopy; and voiding chain cystourethrography.

Table I shows the referral sources of the patient population.

Patients were classified into four groups on the basis of urodynamic studies, and the overall classifications of the 1439 patients are shown in Table II. Since our unit is an integral part of the Multiple Sclerosis Clinic at McMaster University Medical Centre, there was an obvious weighting of this classification. The statistics were adjusted for all neurological referrals (primary neuropathic syndromes, postsurgical neurological trauma, and cerebral vascular syndrome), and the resulting "gynecologic urologic" classifications are shown in Table III. This gynecologic population formed the basis for the present study.

Epidemiologic studies. We examined associations between clinical factors and urodynamic findings to determine the predictive value of the history and examination. The urodynamic findings were used as the "Gold Standard" for the cross analyses with the use of "two-by-two" or fourfold tables (Fig. 1). The results of this cross analysis have been expressed in terms of the predictive value of the symptom or sign relative to the outcome of interest, the urodynamic diagnosis; relative odds express the degree of association between the symptom and the urodynamic diagnosis.

Table III. "Gynecologic" urodynamic classification (1277 patients)

Classification	%
Anatomic incontinence	68
Idiopathic neuromuscular incontinence	22
Mixed-type incontinence	10

Table IV. Major subjective complaints

Complaint	%
Frequency	73
Stress incontinence	66
Urgency	64
Urgency incontinence	44
Nocturia	50
Incomplete emptying	39

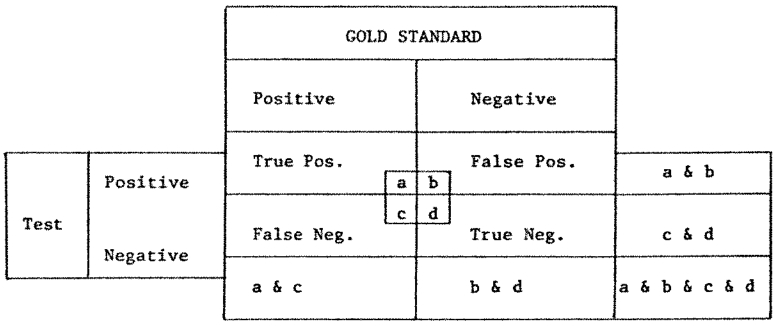
Results

Demographic characteristics. The age span of patients was from 8 to 96 years, with 52% of patients being between the ages of 36 and 55 and 72% between the ages of 36 and 65. The range of parity was from 0 to 13 third-trimester deliveries with 75% of patients having experienced two or more such deliveries.

Of the study population, 57% had a history of previous major gynecologic surgical procedures. Twenty-six percent of the population had undergone previous pelvic operation for urethral-vesical dysfunction and 46% of all major gynecologic operations had been for urinary incontinence. Eighty-three percent of the patients who had such a previous gynecologic-urologic procedure had one or more transvaginal procedures, 15% had a retropubic procedure, and 2% had a combined transvaginal/abdominal urethral-vesical sling.

The commonest associated medical problems were obesity, defined as >2 SD above the mean for the patient's height and age and seen in 31% of the referrals; chronic cardiovascular respiratory disease and diabetes mellitus with their associated medical problems were found in 5% and 3% of patients, respectively. At the time of first consultation, 29% of patients were receiving medications with known neurouropharmacologic effects, the most common being tranquilizers and psychotropic drugs (11%), anticholinergic medication (9%), adrenergic therapy for cardiovascular respiratory disease (7%), and cholinergic therapy (2%).

Clinical characteristics. The major subjective symptoms are recorded in Table IV. In 64% of patients there was clinical evidence on examination of cystocele either alone (32%) or in combination with varying degrees of uterine or vaginal vault prolapse (32%).



		GOLD STANDARD		
		Positive	Negative	
Test	Positive	True Pos. a	b False Pos.	a & b
	Negative	False Neg. c	d True Neg.	c & d
		a & c	b & d	a & b & c & d

Predictive Values:

Positive = a ÷ (a & b)
Negative = d ÷ (c & d)

Relative Odds: $\frac{a \times d}{b \times c}$

= 1 = No Association
= >1 = Positive Association
= <1 = Negative Association

Fig. 1. Predictive value of history and examination.

Table V. Association of clinical presentation and urodynamic diagnoses

	Urodynamic diagnosis					
	Anatomic incontinence			*Neuromuscular incontinence*		
Clinical symptom/sign	*PPV* %	*NPV* %	*RO*	*PPV* %	*NPV* %	*RO*
Stress incontinence, 66%	74	18	12.7	31	69	1.0
Urgency incontinence, 44%	44	95	0.3	66	81	3.8
Stress and urgency incontinence	59	49	1.4	61	29	2.8
Nocturia, 50%	36	67	0.4	46	83	4.4
Cystocele	87	63	10.8	25	65	0.6

PPV = Positive predictive value; NPV = negative predictive value; RO = relative odds.

Table V demonstrates the association, determined by the aforementioned epidemiologic techniques, of an individual's subjective symptom or objective clinical observation of those clinical manifestations and the outcome of interest, the urodynamic diagnosis.

Table VI reveals the association between the history of previous pelvic operation for urinary incontinence and the outcome of interest, the urodynamic diagnosis in patients with such a previous history and now referred with symptoms of recurrent urethral-vesical dysfunction.

Comment

Table VII shows the frequency in the population under study of the major symptoms of urethral-vesical dysfunction as determined by clinical history and the frequency of those same symptoms among the two major categories of diagnostic interest, anatomic sphincteric insufficiency and idiopathic neuromuscular dysfunction.

From the cross-analyses studies it is apparent that many symptoms, although they express urethral-vesical dysfunction, have predictive values that are too low to help in classifying the type of dysfunction.

There are exceptions, as, for example, nocturia, a symptom which predicts idiopathic neuromuscular dysfunction rather than insufficiency of the sphincteric mechanism; however, 39% of patients with urodynamically diagnosed sphincteric insufficiency presented with such a symptom. Also, urgency without incontinence occurs mainly with symptoms of neuromuscular dysfunction, yet 51% of patients with sphincteric insufficiency reported this symptom. Twenty-nine percent of patients whose presenting complaint was

Table VI. Association of history of previous operation for urinary incontinence

	Urodynamic diagnosis					
	Anatomic incontinence			*Neuromuscular incontinence*		
Type of procedure	*PPV (%)*	*NPV (%)*	*RO*	*PPV (%)*	*NPV (%)*	*RO*
Vaginal	68	34	1.1	31	75	1.4
Abdominal	55	44	1.0	38	70	1.5

urgency incontinence urodynamically had sphincteric insufficiency, and 17% of patients who complained of stress incontinence had urgency incontinence.

An isolated complaint is strongly predictive of the final diagnosis. The complaint of stress incontinence strongly predicts sphincteric insufficiency and the complaint of urgency incontinence similarly predicts idiopathic neuromuscular incontinence.

The dilemma for the gynecologist is that the meaning of the symptoms is an enigma in many patients, particularly when there is a multiplicity of subjective complaints. In this study of 1277 patients with "gynecologic" urethral-vesical dysfunction, 68.2% had anatomic sphincteric insufficiency and 22.2% had idiopathic neuromuscular incontinence. However, of the patients who complained of urgency incontinence, on urodynamic study, 29% had genuine stress incontinence; as a corollary, among patients who complained of stress incontinence, 17% of patients had motor instability and dyssynergia of the detrusor muscle. When the symptoms of both stress and urgency incontinence coexisted in the same patient, urodynamic studies revealed that 53% of patients had anatomic incontinence and 37% had neuromuscular incontinence.

In summary, gynecologic patients presenting with symptoms of urethral-vesical dysfunction, that is, symptoms of urgency, nocturia, urgency incontinence, and stress incontinence, each symptom taken in isolation is positively predictive of the major form of dysfunction. Anterior vaginal wall relaxation with or without prolapse is a positive predictive factor of loss of vesical neck support, predisposing to sphincteric insufficiency.

Table VII. Symptoms of urethral-vesical dysfunction (1277 patients)

	Urodynamic diagnosis	
Symptom	*Anatomic (%)*	*Neuromuscular (%)*
Frequency, 73%	63	88
Nocturia, 50%	39	73
Urgency, 64%	51	84
Urgency incontinence, 44%	29	65
Stress incontinence, 66%	88	17
Incomplete emptying, 39%	29	37

The more complex the clinical presentation, the more difficult the diagnosis of the underlying pathophysiologic state. In such patients urodynamic investigation and diagnosis of the clinical presentation are of value in the establishment of an accurate urodynamic diagnosis and of a rational plan for therapy.

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The Marlex sling operation for the treatment of recurrent stress urinary incontinence: A 16-year review

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Between 1968 and 1983, 281 patients with recurrent stress urinary incontinence and nine patients with primary stress urinary incontinence were treated with a two-team (suprapubic vaginal) Marlex urethral sling operation. Selection was based on the patient's history and the clinical demonstration of an anatomic defect in the closure mechanism of the urethra. The operative technique as described in 1970 remains unchanged. The bladder neck is released from the scar and replaced in a midretropubic position on a hammock of Marlex mesh attached to Cooper's ligaments. Patients with a sloughed urethra had a sling operation in conjunction with the reconstruction of a neourethra. The overall success rate based on a minimum 5-year follow-up of 208 patients was 77.4%. There was a high failure rate in the sloughed urethra group. Significant overall complications include bladder neck obstruction and chronic cystitis. (AM J OBSTET GYNECOL 1985;151:224-6.)

Key words: Bladder, urethra, Cooper's ligament, cystometry, uretropexy

Recurrent stress urinary incontinence following primary anterior vaginal repair has been traditionally treated in North America with a suprapubic urethropexy of the Marshall-Marchetti-Krantz or Burch type. Failure of these secondary procedures is due to either breakdown or scar. Fixation of the base of the bladder in the retropubic space or to the anterior vaginal wall, often results in an incontinent "drainpipe" urethra. For these patients, a sling operation is required. A key to the surgical success with any sling procedure is the complete release of the base of the bladder and the urethra from a bed of scar, followed by repositioning of the bladder neck in an intra-abdominal retropubic position. This may be safely accomplished by a two-team approach with a broad hammock of Marlex mesh used to suspend the bladder neck from Cooper's ligaments.

Material and methods

From January 1, 1967, to December 31, 1983, 281 patients with recurrent stress urinary incontinence and nine patients with primary stress urinary incontinence were treated with a two-team Marlex urethral sling operation.

Of these, 103 had a previous failed anterior colporrhaphy and 13 a failed primary Marshall-Marchetti operation.

In addition, two to five unsuccessful continence operations were performed in 149 patients, of whom 76 had a vaginal repair followed by a Marshall-Marchetti operation, 30 had two vaginal repairs, 32 had three operations consisting of both vaginal and suprapubic procedures, nine had four operations, and two had five or more such procedures.

Nine patients had a primary two-team sling operation because of vaginal vault fixation following abdominal or vaginal hysterectomy or because of chronic cough due to chronic chest disease.

Finally, 16 patients who presented with a totally sloughed urethra had a Marlex sling operation performed immediately following reconstruction of a neourethra from the anterior vaginal wall insulated by a Martius bulbocavernosus graft.[1]

Selection was based entirely on a precise history and a clinical demonstration of an anatomic defect in the closure mechanism of the urethra. Demonstration of restored continence with gentle lateral vaginal fornix elevation of the bladder neck in lithotomy or standing position with a bladder volume of 100 ml was accepted as evidence for the potential of the sling. Urethral pressure studies done laterally did not influence the decision. All patients had cystoscopy, urethral panendoscopy, and either water or carbon dioxide cystometry to confirm detrusor stability. Findings suggesting detrusor instability did not exclude patients from operation if a urethral closure defect was evident and considered the major cause of both the stress incontinence and the detrusor instability.

Lateral cystourethrograms with the three-contrast technique of Low[2] precisely defined the anatomic defect in the urethra and, most important, the position of the

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bladder neck in relation to the posterior surface of the symphysis pubis (Fig. 1). A high bladder neck often signaled increased technical difficulty in releasing the scar. Such studies also revealed five unsuspected urethral diverticula and two fistulas of the urethra.

The technique, as described in 1970, remains unchanged.[3] The vaginal and abdominal teams working simultaneously, assisting each other under direct vision, completely release the bladder and urethra. Wide tunnels are made through the scarred fascia of the perineal membrane after which the bladder and urethra are rendered totally mobile. The bladder neck is then replaced in a midretropubic position, lying on a 1 to 2 cm band of Marlex mesh suspended from Cooper's ligaments (Fig. 2). Minimal tension is demonstrated at the urethral-vesical junction by the ease with which a standard-size transurethral Foley catheter slides in and out of the bladder neck. The operation takes less than 2 hours. Postoperative suprapubic catheter drainage was routine. Normal voiding patterns were usually established by the tenth postoperative day and most suprapubic catheters were removed 12 to 14 days after the operation.

Results

Two hundred eight patients operated on between 1967 and 1979 were interviewed personally or contacted by letter or telephone; results were based on the follow-up of a minimum of 5 years (Table I). Nineteen patients could not be traced. The overall success rate of 77.4% rises to 80.9% if the 14 sloughed urethras and their 10 failures are excluded.

In 82 patients recently operated on (1979 to 1983) who were followed up from 1 to 4 years, the overall success rate to date is 92.7% (Table II).

Those patients who said they no longer required protective pads and who were able to resume normal activity without stress incontinence were considered operative successes.

Complications. There were no deaths. No slings have been rejected or removed. In 10 of 16 patients in the sloughed urethra group, an edge of Marlex mesh had pulled into the neourethra and Martius graft resulting in urethral stone formation. The Marlex mesh was cut and removed transurethrally. The stones were removed, but all patients remain incontinent. To date, two have ileoconduits.

In the main group (274 patients), two slings have pulled through the urethra, one after 3 years, the other 1 year after a repeat sling operation. In both instances the mesh was recently cut transurethrally and, although they remain continent, the result is uncertain. Two additional patients have required urinary diversion because of severe chronic cystitis and end-stage contracted or systolic bladder.

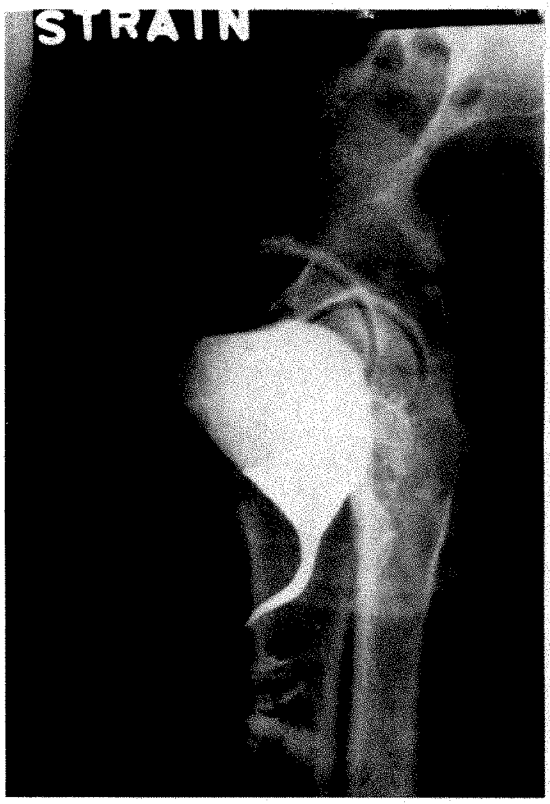

Fig. 1. Straining lateral cystourethrogram of patient with previous vaginal repair and Marshall-Marchetti operation shows fixed drainpipe urethra.

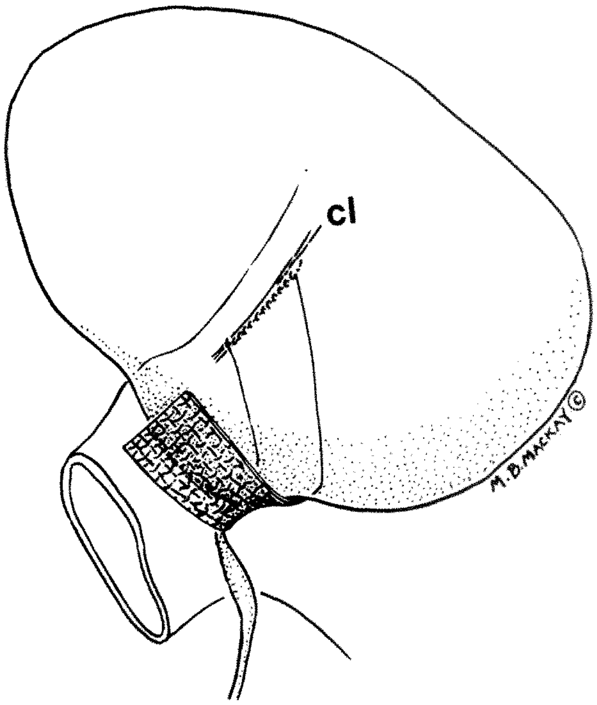

Fig. 2. Strip of Marlex mesh suspends the bladder neck from Cooper's ligaments *(cl)*.

Table I. Results of 5-year follow-up of 208 patients (1967 to 1979)

Previous operations	*Total*	*Success*	*Failure*	*No. lost to follow-up (considered failure)*	*% Success*
1	84	71	13	5	84.5
≥2	120	86	34	14	71.7
Primary sling	4	4	0	0	100
Total	208	161	47	19	77.4

Table II. Results of 1- to 4-year follow-up of 82 patients (1979 to 1983)

Previous operations	*Total*	*Success*	*Failure*	*No. lost to follow-up (considered failures)*	*% Success*
1	32	29	3	0	90.6
≥2	45	42	3	0	93.3
Primary sling	5	5	0	0	100
Total	82	76	6	0	92.7

Prolonged postoperative urinary retention was rare. Twelve patients with persistent outlet obstruction and urinary retention required anterior transurethral resection of the bladder neck. Two patients catheterize themselves routinely. Ten patients have recurrent cystitis related to poor bladder empyting.

That significant wound infection was rare is attributed to the suprapubic catheter, which was an efficient drain. Six patients had large hematomas requiring drainage. Three had chronic catheter sinus tracts that were eventually excised. Although urgency and frequency unrelated to demonstrable urinary tract infection were common early postoperative complications, they persisted in only 5% of patients.

Comment

Clinical parameters continue to provide the most reliable assessment in the diagnosis of recurrent stress urinary incontinence. We have not performed urethral flow and pressure studies in patient selection or diagnosis. A cystometrogram, however, is essential to rule out an unstable bladder. A lateral cystourethrogram precisely defines the urethral defect, the position of the bladder neck, and the degree of pelvic floor scarring. The two-team approach facilitates the operation, allowing wide mobilization under direct vision, minimizing blood loss, and eliminating urinary tract injury. With experience, we have determined that a high retropubic placement of the bladder neck is not necessary, thus diminishing the incidence of postoperative bladder neck obstruction and retention. This may account in part for the improved results since 1979.

Excellent results with primary sling suggest that this operation should be considered more often than a primary vaginal repair or primary retropubic urethropexy in patients who are at high risk because of hysterectomy scar or chronic chest disease.

Women with recurrent stress urinary incontinence following two or more unsuccessful operations have been termed salvage cases, implying poor results with further operation. In this series, the overall success rate in this group at 5 years was 86 out of 120 (71.9%).

These figures do not include as successes any patients who died or were lost to follow-up before 5 years. The actual success rate may therefore be marginally better than reported. The unacceptable high failure rate in the sloughed urethra group suggests that, following construction of a neourethra with bulbocavernosus graft, a technique of support other than an immediate suburethral Marlex sling must be developed.

Certain patients who have had three or more previous operations, although continent, have ongoing voiding problems. Some may have a partially denervated bladder. Although results in this group of patients are only fair, the Marlex sling operation may be an acceptable alternative to total urinary incontinence or final urinary diversion.

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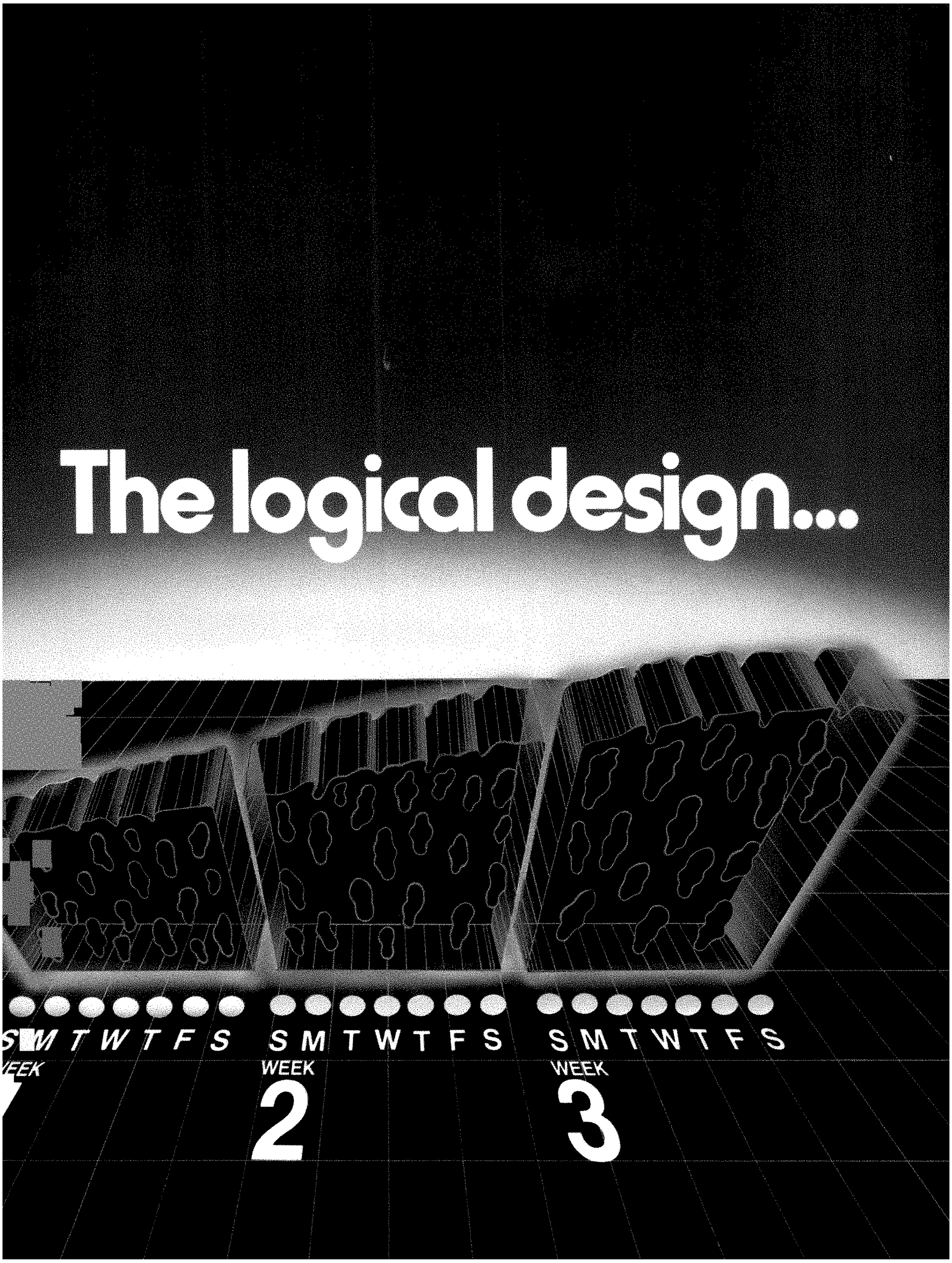
The logical design...
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The logical choice...

for low breakthrough bleeding[†] in each phase of the cycle

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with week-by-week simplicity

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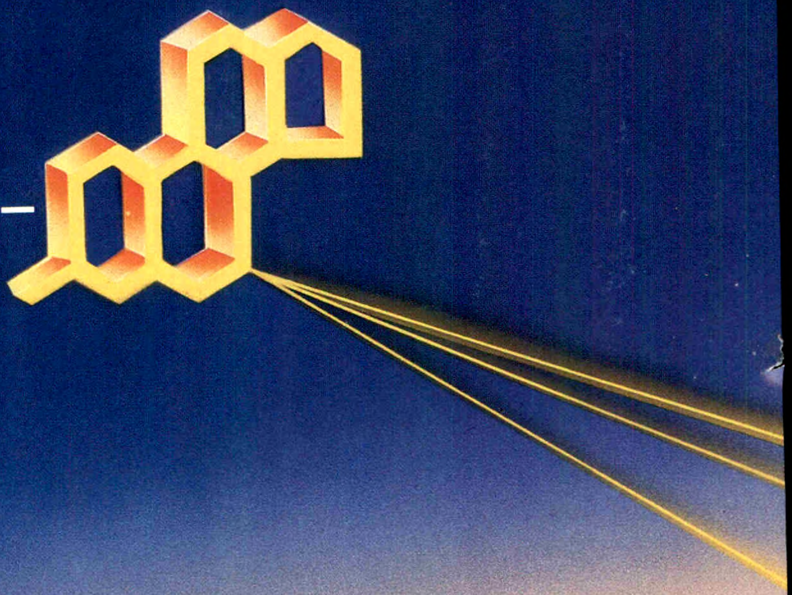

[†]Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

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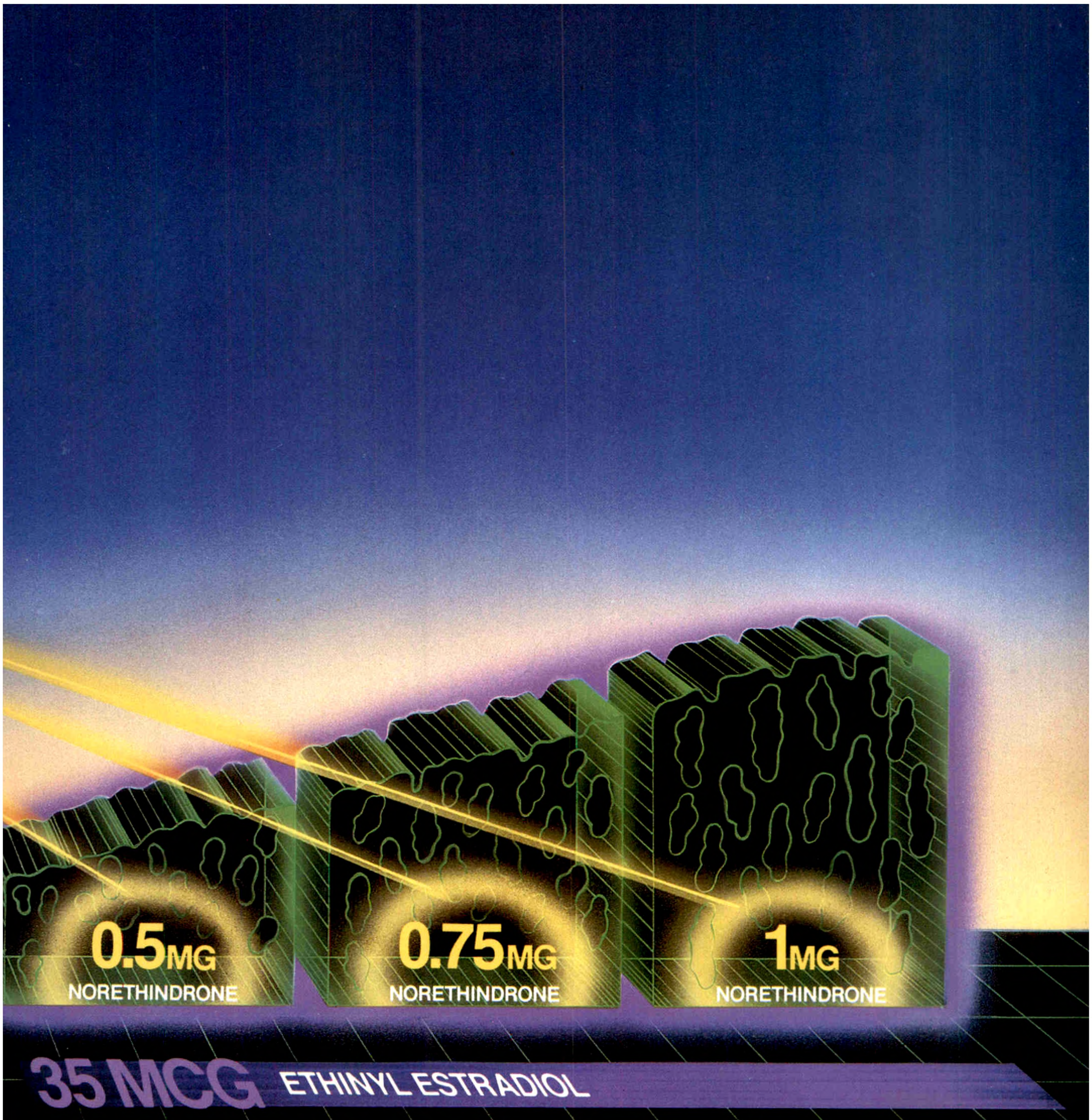

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IDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

OSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a ositive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For is reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The al contraceptive product prescribed for any given patient should be that product which contains the least amount estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that ew acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

ONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1 hrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembo- c disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5 nown or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contracep- ve tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are ontraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient ould be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor hich developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and rombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great ritain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous romboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral ontraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall xcess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 sers and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrova- cular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic troke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for ll cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years fter last oral contraceptive use. A prospective study conducted in the United States found that past use of oral ontraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There as also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **YOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contracep- ves has been reported confirming a previously suspected association. These studies, conducted in the United ingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease igarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the igher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user r not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate ncreased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be pproximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in e 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke moking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a tal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have bout a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold creased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important ctor. In determining the importance of these relative risks, however, the baseline rates for various age groups ust be given serious consideration. The importance of other predisposing conditions mentioned above in etermining relative and absolute risks has not as yet been quantified. It is quite likely that the same synergistic ction exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in al contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual sk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national dverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including oronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 00 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 cg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor volved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the egree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. ases of thromboembolic disease have been reported in women using these products, and they should not be esumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and onusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor r these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study arried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the rculatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of se. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to e 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being oncentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, owever, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of ontinuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory iseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of omen under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude f the relative risk for this younger age group. This study reports that the increased risk of circulatory disease ortality may persist after the pill is discontinued. Another study published at the same time confirms a previously eported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality ssociated with all methods of birth control is low and below that associated with childbirth, with the exception of al contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for mokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive se in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest ortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and hrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for yocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of reeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive sers is substantially increased in women age 40 and over, especially those with other risk factors. The physician nd the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., hrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, nd mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued mmediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in ral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery f a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS here have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use f oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial r complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute ppropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either atural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, agina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted o increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control tudies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous strogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a egistry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases ound in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding t the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a equential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting n increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contracep- ives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. n summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Angiographic arterial embolization in the management of postoperative vaginal hemorrhage

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Recently great success was achieved at the Toronto General Hospital in treating patients with postoperative vaginal hemorrhage by angiographic methods. A retrospective review was performed from 1975 to 1984 on eight patients treated by this technique for nine separate episodes of hemorrhage with a mean estimated blood loss in excess of 5000 ml. Success was achieved in seven episodes despite failure of various vaginal and abdominal approaches. One of the failures was due to previous bilateral hypogastric artery ligation that made the bleeding branch inaccessible to the angiography catheter. The other patient developed bleeding following cesarean section which did not respond to angiographic embolization due to faulty technique. Both of these patients were eventually cured by abdominal surgery. We feel that angiographic embolization is the procedure of choice in the management of postoperative vaginal hemorrhage when conservative management has failed or is inappropriate; it can be lifesaving when all other attempts to stop the hemorrhage have failed. (AM J OBSTET GYNECOL 1985;151:227-31.)

Key words: Angiographic embolization, postoperative vaginal hemorrhage, abdominal surgery

Angiographic techniques have recently found great application in the management of hemorrhage in obstetrics and gynecology. Many case reports have recently been published on the use of angiography in controlling bleeding from gynecologic malignancies,[1-3] menorrhagia,[4] as well as in the treatment of postabortal,[5-7] postpartum,[8-10] and postoperative hemorrhage.[11, 12] In the past, angiography was only attempted as a last resort in patients who were considered poor surgical risks; with increasing success and experience the confidence in angiography has grown to the point where angiographic embolization can be considered an integral part in the management of any patient with postoperative vaginal hemorrhage.

The present study reports our experience with angiographic techniques in the control of postoperative vaginal bleeding, and it further emphasizes the previous report comparing angiographic with more traditional methods.[11] The overall results suggest that failures and complications of angiographic arterial embolization can be minimized if a rational approach is followed and proper technique is used.

Material and methods

Since 1975 in the Toronto General Hospital, eight patients were treated by angiographic techniques for significant postoperative vaginal hemorrhage in nine separate episodes of bleeding. The profiles of these patients are shown in Table I. As is often the case, all primary gynecologic surgery was performed for benign disease in relatively young and healthy women. Unfortunately each suffered from excessive postoperative vaginal bleeding, with shock and coagulation disorders in many. Only Case 3 could be considered at high risk because of rheumatic heart disease, requiring mitral valve replacement and use of anticoagulants. All except Case 2 were referred from surrounding peripheral hospitals for tertiary care. Cases 7 and 8 had persistent postoperative vaginal bleeding following cesarean section from a bleeding arterial vessel in the lower uterine segment incision.

Angiography consultation is available on a 24-hour basis at the Toronto General Hospital, and referrals are encouraged from other hospitals where this service and expertise are not available. Ancillary facilities such as blood banks, intensive care monitoring, and other surgical and medical specialists are readily available for assistance, which provide the angiographer with the time and confidence so necessary for a successful result.

The actual procedure involves Seldinger puncture of the femoral artery under local anesthesia in an awake patient. This technique is especially applicable to pelvic hemorrhage in young women with minimal vascular disease because of the easy and direct access to the bleeding branches of the hypogastric artery by approaching the femoral artery on the *opposite* side.

The technical procedure should include the following series of steps[13]: (1) a baseline aortogram is taken to outline the hypogastric arteries bilaterally and iden-

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Table I. Patient summaries

Case	*Age (yr)*	*Primary surgery*	*Indication*	*Estimated blood loss (ml)*
1	48	Abdominal hysterectomy	Menorrhagia	2,000
2	39	Abdominal hysterectomy	Menorrhagia	1,600
3a, 3b	46	Abdominal hysterectomy	Menorrhagia	10,800
4	42	Abdominal hysterectomy	Ovarian cyst	12,800
5	29	Vaginal trachelectomy	Cervicitis	6,400
6	38	Vaginal hysterectomy	Menorrhagia	2,000
7	22	Cesarean section	Disproportion	6,400
8	29	Cesarean section	Breech	8,000

Table II. Treatment methods

Case	*Vaginal treatment*	*Abdominal treatment*	*Angiography*	*Outcome*
1	Packing; suture of vault	Suture of vault; bilateral hypogastric artery ligation	Balloon catheter and Pitressin infusion of right hypogastric artery*	Pulmonary congestion; prolonged hospitalization; wound infection
2	Packing	—	Embolization of branch of left hypogastric artery (coil)*	Good
3a	Packing; suture of vault	Suture of vault	Embolization of left hypogastric artery (4 coils)*	Good until heparin restarted
3b	Packing	—	Embolization of right hypogastric artery (coil)*	Good after heparin restarted
4	Packing; suture of vault	Suture of vault; bilateral hypogastric artery ligation; vascular surgery*	Abandoned since arteries were ligated	Good; multiple transfusions
5	Packing	—	Embolization of branch of left hypogastric artery (coil)*	Good
6	Packing; suture of vault	—	Embolization of right hypogastric artery (Gelfoam + 5 coils)*	Gluteal pain due to refluxed coil
7	—	Suture of incision	Embolization of branch of left hypogastric artery (coil)*	Wound infection; coagulopathy
8	Dilation and curettage	Suture of incision; hysterectomy*	Embolization of branch of left hypogastric artery (coil)	Good

*Denotes successful treatment.

tify the bleeding branch; (2) the tip of the catheter is advanced distally toward the arterial bleeding site to maximize hemostasis and minimize ischemia to adjacent tissues following embolization; (3) the artery can then be embolized with any number of materials[14]—most commonly used is gelatin sponge (Gelfoam), but the authors have had great success with the Gianturco (new) coil[15] and have found it to be ideal for a bleeding terminal artery with little need for restoring vascular continuity; (4) a postembolization film is taken to ensure obstruction of the vessel; (5) the catheter is then withdrawn and inserted into the contralateral hypogastric artery to ensure that one of its branches is not bleeding or supplying a bleeding collateral vessel; (6) a postembolization aortogram is mandatory to demonstrate no further extravasation.

The entire procedure can be completed in considerably <2 hours by a skilled angiographer in most cases.

Analysis of results

The clinical course of each of the patients is outlined in Table II. Cases 1 through 4 were treated by angiographic methods for five episodes of hemorrhage following abdominal hysterectomy, with success achieved in four. Of interest are Cases 1 and 4 for whom previous vaginal and abdominal attempts had failed to control

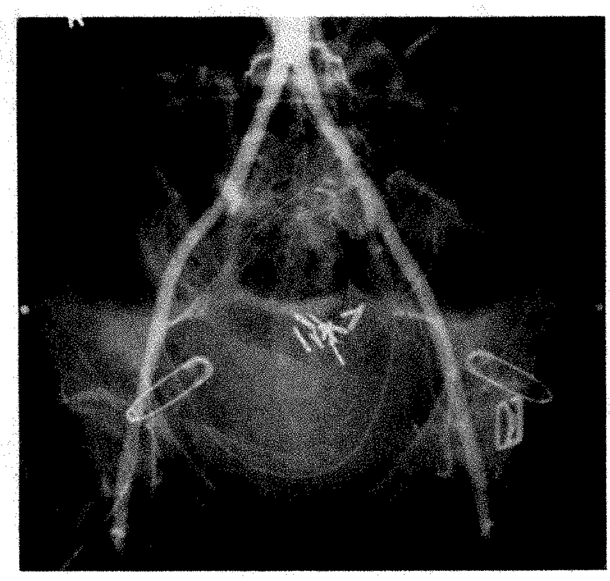

Fig. 1. Aortogram demonstrating filling of the distal branch of the left hypogastric artery *(arrow)* despite previous ligation.

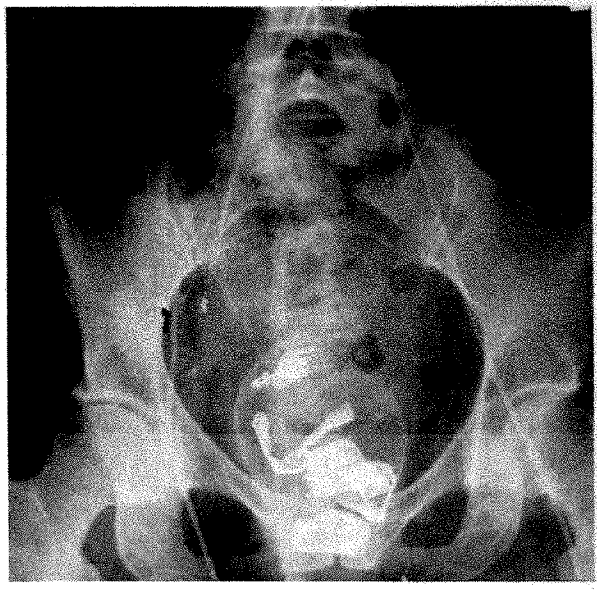

Fig. 2. Film showing multiple coils in proper position in the anterior division as well as refluxed coil in the posterior division *(arrow)* of the right hypogastric artery.

the persistent hemorrhage. Angiography was successful for Case 1 because of a patent right hypogastric artery despite previous bilateral surgical ligation. This allowed the passage of a balloon catheter and infusion of vasopressin into the bleeding branch, which was lifesaving for that patient and heralded the use of this technique for other bleeding patients. The method itself was cumbersome and associated with complications and was therefore replaced with embolization in future cases.

Case 4 also underwent angiography following bilateral hypogastric artery ligation, but unfortunately the catheter could not reach the bleeding distal to the surgical ligature (Fig. 1). Therefore exhausting and difficult vascular surgery was necessary to ligate the distal branches of the left hypogastric artery, which was successful in controlling the bleeding.

Case 3 was at high risk because of her prosthetic mitral valve and anticoagulants. Selective angiographic arterial embolization controlled the bleeding from the branch of the left hypogastric artery despite failure of vaginal and abdominal approaches. Contralateral angiography was not performed as described in the technical portion of the previous section. The bleeding stopped until initiation of heparin therapy postoperatively. Rather than use of surgery, angiography was repeated and the bleeding from the opposite side was controlled by embolization. It was felt that these were separate bleeding episodes at separate sites and signified two successes of angiographic embolization rather than a failure. It is unlikely that the contralateral angiogram would have demonstrated extravasation, since there was no bleeding clinically for 48 hours.

Embolization techniques were successfully utilized instead of abdominal approaches in Cases 5 and 6, who sustained hemorrhage following vaginal operations. Unfortunately, Case 6 developed temporary right gluteal muscle pain following reflux of one of the coils into the posterior division of the right hypogastric artery (Fig. 2). The symptoms slowly resolved over 7 days with conservative treatment.

Cases 7 and 8 were included in this study because both had persistent severe vaginal hemorrhage following cesarean sections despite conservative attempts which included intravenous oxytocin and antibiotics. Angiography was attempted following various failures of vaginal and abdominal surgery. Embolization was successful in Case 7, which also preserved her fertility. Case 8 was not as fortunate and eventually required a hysterectomy to control the bleeding.

Comment

Postoperative vaginal hemorrhage presents a great challenge to any surgeon. He is indeed fortunate to have the benefit of skilled angiographers at his disposal, who may have better access to the bleeding artery than the surgeon who would ligate it.[16, 17] Despite the simplicity of the technique, success rates will vary depending on the experience and skill of the angiographer. In order to achieve success and safety, it is necessary to adhere to proper techniques and have adequate ancillary facilities available.

The only true failure of embolization occurred in Case 8, who was still bleeding following the procedure. The postembolization film showed occlusion of the bleeding branch of the left hypogastric artery, but a contralateral hypogastric arteriogram and aortogram were omitted and the bleeding site was therefore not identified. This unfortunate patient required a hysterectomy, which might have been prevented with stricter adherence to technique.

Advantages of angiography over surgery include the ability to localize the bleeding site in the majority of cases. It is then possible to provide a more distal occlusion than surgical ligation. The surgical option is still open for failures of embolization without having compromised the patient. Success or failure will soon be evident by clinically observing the patient's bleeding.

Risks of the procedure in controlling pelvic hemorrhage are minimal in skilled hands. Technical problems related to the arterial catheterization and embolization should be virtually nonexistent in relatively young and healthy women.[15] Ischemic complications such as fever, pain, and tissue necrosis must be considered in patients with vascular compromise such as those with atherosclerosis, extensive radical surgery, or previous radiotherapy.[18, 19] Angiography is still useful for such patients, but caution is advised in order to preserve the collateral circulation to surrounding organs. High flow rates in bleeding branches of the hypogastric artery tend to protect against reflux of the embolus.[2] This complication occurred in Case 6 because the bleeding artery was converted to a low-flow system by packing it with Gelfoam prior to embolizing it with coils. Thus injection of additional emboli into an embolized vessel permits reflux and should be performed with extreme caution.

Radiation exposure and renal toxicity of angiographic dyes are potential risks that the angiographer should be aware of.

Various types of embolization material are available. Gelfoam is most commonly used because of its availability and resorption capability allowing restoration of vascular integrity. This might be important in the embolization of bleeding organs, but the Gianturco coil has been found to be suitable for bleeding from a terminal artery in which a ligature has slipped.

A rational approach to the management of patients with postoperative vaginal bleeding should therefore include the following considerations:

1. Resuscitation. Hypovolemia should be treated with fluid replacement and infusion of blood and the appropriate blood products. Intensive care monitoring and coagulation studies are performed as clinically indicated.

2. Examination. Specific attention should be directed to the amount and location of the bleeding, which will guide the angiographer in his approach to the appropriate femoral artery.

3. Vaginal approach. A vaginal approach is appropriate if bleeding is from an artery at the vaginal vault which can be ligated. Vaginal packing is useful for venous oozing but should be abandoned if the bleeding is brisk or the packing is already soaked.

4. Angiographic embolization. Embolization should be considered early in the course of events while the coagulation status and vital signs are relatively normal. It is safe, simple, and effective and will eliminate major surgical procedures for the great majority of patients. In the event of failed embolization, serious consideration should be given to a repeated attempt at angiography if clinically appropriate.

5. Abdominal surgery. Surgery should be reserved for failures of angiographic methods. It should be emphasized that repeated surgery can be extremely difficult because of edematous tissues, distorted anatomy, and lack of familiarity with the retroperitoneal space. The risk of damage to adjacent structures is significant. One can question the added effectiveness of bilateral hypogastric artery *ligation* following bilateral hypogastric artery *embolization.*

Angiography following hypogastric artery ligation can create technical difficulties that are not insurmountable. This situation has been successfully managed by identifying the collateral vessel feeding the distal hypogastric artery and embolizing it[8, 9] or infusing vasopressin into it.[20] In retrospect, Case 4 might have been managed in this manner and further surgery might have been prevented.

In conclusion, angiographic arterial embolization has proved itself to be a most useful clinical tool in the management of postoperative vaginal hemorrhage. It should prevent needless procrastination by the surgeon who is reluctant to reoperate on a compromised bleeding patient. A skilled angiographer should be consulted *early* in the course of events to assist in the management of these patients. If principles are adhered to, a high success rate with minimal morbidity should follow, thereby preventing major abdominal surgery in a great majority of cases.

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Perinatal factors associated with death or handicap in very preterm infants

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That death or major developmental handicap is associated with prematurity and low birth weight is well recognized. The importance of other perinatal factors related to presentation and management, however, is far from certain. In an attempt to elucidate the importance of some of these factors, data from 383 live-born infants delivered at 26 to 30 weeks' gestation were analyzed. All infants were born in a tertiary perinatal unit and long-term follow-up had been carried out on the survivors for at least 1 year. A group of 39 infants who died in the neonatal period and 34 infants with long-term handicap were compared with matched normal control infants. Perinatal factors related to outcome were analyzed and it was found that, while initial poor condition at birth was correlated with death, there were few predictors of subsequent handicap. More refined methods of both prenatal and neonatal assessment are required to define these factors. (AM J OBSTET GYNECOL 1985;151:231-8.)

Key words: Perinatal death, long-term handicap, very preterm infant

It has been realized for many years that infants delivered prematurely are associated with an increased occurrence of neonatal mortality as well as an increased chance of long-term handicapping conditions, compared to infants born at term.[1] These are the result of immature physiologic mechanisms for dealing with the requirements of extrauterine existence, demonstrated by difficulties in respiratory control and gas exchange as well as immaturity in many other organ systems.

Long-term handicaps are primarily the result of insults occurring in the immature brain, either by hemorrhage from poorly supported blood vessels or

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Table I. Handicaps defined in damaged survivors

Handicap	*No. of damaged survivors*
Cerebral palsy	
Mild to moderate motor disability, normal intelligence (normal schooling)	15
Severe motor disability, with or without blindness, normal intelligence (special schooling)	6
Any degree of motor disability plus mental retardation (special schooling)	6
Mental retardation (normal motor skills)	5
Behavior problems	1
Hydrocephalus (normal development)	1

through ischemic damage, hypoglycemia, or other adverse factors.[2-4]

Nevertheless, with the advances in perinatal care, both obstetric and neonatal, the outlook for prematurely born infants has improved dramatically over the past two decades.[5, 6] There is a need to attempt to define those adverse factors that increase the chances of death or handicap in this group of infants, since the mechanisms involved in the pathogenesis of many of these are poorly understood.

Many of our current approaches to the perinatal management of prematurity may not be able to stand up to close scientific scrutiny. Among these are the use of cesarean section in the premature breech and the administration of tocolytic agents for the delay or prevention of premature labor.[7, 8]

There has been a realization that the measurement criteria of improvements in perinatal care should no longer be confined to those defined in mortality statistics but should include some attempts to ascertain morbidity outcomes.[9, 10] Indeed, this has not been a belief confined to perinatology but has become an integral part of the cost-effectiveness assessment of many programs in health care. Until recently, the development of adequate follow-up programs for the assessment of high-risk newborn infants has been delayed by the emphasis on providing the service components of high-risk perinatal care.

Several authors have raised serious concerns regarding the appropriateness of the provision of perinatal care to extremely low–birth weight infants. These concerns have been related to cost-benefit considerations as well as morbidity suffered by a significant proportion of these infants.[11-13] Data are not readily available, however, to assist in defining groups in whom the major gain may be obtained by the application of currently available knowledge and technology.

Table II. Factors analyzed for each study infant

- Pregnancy
  - Predelivery time in unit
  - Pregnancy complications (premature labor, premature rupture of membranes, antepartum hemorrhage, etc.)
  - Singleton or twin
  - Preexisting maternal disease (for instance, diabetes, hypertension)
- Delivery
  - Vaginal or cesarean section
  - Membranes intact or not intact
  - Antenatal betamethasone therapy (time before delivery >36 hr)
  - Fetal heart rate, normal or abnormal
  - Type of anesthesia
- Infant
  - Weight, gestational age
  - Appropriate for gestational age or small for gestational age
  - Apgar score
  - Cord vein pH and base deficit
  - Assisted ventilation and diagnosis
  - Pneumothorax
  - Duration of assisted ventilation (intermittent positive pressure ventilation or continuous positive airway pressure)
  - Duration of oxygen therapy
  - Maximum F_{IO_2}, pressure, and rate
  - Patent ductus arteriosus
  - Lowest blood pressure (0-72 hr, hourly measurements)
  - Highest blood pressure
  - Need for plasma transfusion
  - Subependymal or intraventricular hemorrhage (grade)
  - Time to diuresis (output exceeding input)
  - Infection
  - Initial hemoglobin, lowest and highest white blood cell count
  - Platelet count (<100,000/mm^2)

The present study is an attempt to define factors associated with poor outcome in a group of premature infants born in a single tertiary care perinatal unit.

Material and methods

The study population was taken from all infants born in the Perinatal Unit of Women's College Hospital at an obstetric gestation of <32 weeks, between January 1, 1979, and December 31, 1982.

In total, there were 730 live-born infants during that period of time.[11] All infants with congenital anomalies incompatible with life were excluded. The study group was further defined by exclusion of infants less than 26 weeks and more than 30 weeks of gestational age.

The reasons for these exclusions were to concentrate the analysis on those infants whose perinatal management possibly would not have been compromised by borderline gestation and to include only those who had been personally evaluated in our follow-up program.

This left a group of 383 live-born infants with gestations from 26 to 30 weeks at delivery, all of the survivors having been followed up in the Clinic for at least 1 year.

In this population, there were 39 infants who did not survive and 34 infants who were considered to dem-

Table III. Comparison of prenatal and delivery data (39 dead infants versus 39 control infants)

Factor	*Dead infants*	*Control infants*	χ^2	*p*
Premature labor	9	18	3.625	0.034*
Premature rupture of membranes >24 hr	19	16	0.207	0.79
Antepartum hemorrhage or other	11	14	1.58	0.144
Predelivery time in Unit <1 day	30	17	7.709	0.00304†
Singleton	28	32	0.65	0.358
Twin	11	7	0.65	0.358
Vertex	23	20	0.207	0.79
Breech	15	12	0.227	0.747
All complications	13	10	0.246	0.711
Vaginal delivery	14	12	0.0577	1.614
Cesarean section	27	27	0.060	1.58
Membranes intact	16	15	0.0	—
Fetal heart rate normal	17	30	7.709	0.00304†
Betamethasone therapy >36 hr	11	21	4.292	0.0225*
General anesthesia	23	22	0.0	—
Epidural anesthesia	15	11	0.659	0.354
Male/female	24/15	14/25	4.157	0.025*
Small for gestational age	13	6	2.505	0.072

*p < 0.05.
†p < 0.005.

onstrate significant handicap at follow-up. These infants were all seen by one of the authors (E. M. H.) and were considered to be handicapped if a motor, mental, or combined deficit was discovered that was sufficiently severe to be expected to interfere with normal functioning.[6] The list of handicaps found is shown in Table I.

The dead and handicapped infants were paired with the infants born closest to their dates of birth with the same gestational ages who did not demonstrate abnormality at follow-up.

There were, therefore, 146 infants included in the detailed analysis, 39 dead plus 39 control, and 34 handicapped plus 34 control. The same control infants were not used for both dead and handicapped groups. Obstetric and neonatal variables were recorded for each infant and these data were coded and analyzed. The data recorded for each of the study infants are shown in Table II.

Statistical analysis was carried out by Student's *t* test for numerical data and χ^2 analysis with the Yates correction for incidence data.

Results

The mean gestational ages and weights of the groups were thus not significantly different, demonstrating adequate control selection. The group of dead infants had a mean weight at birth of 960 ± 264 gm and a gestation of 27.6 ± 1.6 weeks, compared to 1021 ± 239 gm and 27.5 ± 1.3 weeks in the control infants.

The group of handicapped infants had a mean weight of 1078 ± 208 gm and a gestation of 28.2 ± 1.0 weeks, compared to 1032 ± 184 gm and gestations of 28.1 ± 1.2 weeks for the control infants.

Dead infants versus control infants. The results of the analysis of prenatal delivery factors in the group of dead infants versus control infants are shown in Table III.

Fewer of the dead infants had premature labor as a complication. Other complications, however, such as prolonged rupture of the membranes, antepartum hemorrhage, maternal diabetes, toxemia, or hypertension, were not seen more commonly in the dead group compared to the control group.

The presentation of the fetus, mode of delivery, and type of anesthesia used were not different in the groups. However, a period of time in the Perinatal Unit of <1 day, an abnormal fetal heart rate pattern prior to delivery, and a course of antenatal betamethasone of <36 hours' duration were all more common in the dead group.

The state of the membranes at delivery (ruptured or intact) was not different in the dead and control groups.

Smallness for gestational age, diagnosed either prior to delivery or afterward, was slightly more common in the dead group than in the control group (13 of 39 vs. 6 of 39, $p < 0.10$).

There was a slight increase in the proportion of male infants who died.

The condition at birth of the group of dead infants versus the control infants is shown in Table IV.

There were significant reductions in both 1- and 5-

Table IV. Numerical neonatal factors in dead infants versus control infants (±SD)

Neonatal factor	*Dead infants*	*Control infants*	*T*	*p*
Mean gestation (wk)	27.6 ± 1.6	27.54 ± 1.33	0.078	0.97
Mean weight (gm)	960.5 ± 264.0	1020.8 ± 239.4	1.055	0.148
Apgar score at 1 min	3.8 ± 2.4	4.9 ± 2.2	2.226	0.124
Apgar score at 5 min	6.6 ± 2.4	8.5 ± 3.5	3.064	0.0017*
Cord venous pH	7.26 ± 0.14 (n = 31)	7.34 ± 0.09 (n = 27)	2.58	0.006†
Cord base deficit	8.2 ± 5.4	4.8 ± 3.8	2.724	0.0042*
Duration of assisted ventilation (days)	3.2 ± 2.9	7.8 ± 10.2	N/A	—
Duration of oxygen therapy (days)	3.2 ± 2.9	31.8 ± 23.9	N/A	—
Maximum oxygen (mm Hg)	88.8 ± 15.9	50.8 ± 16.2	N/A	—
Maximum pressure (cm H_2O)	27.2 ± 5.7	20.3 ± 5.7	N/A	—
Maximum rate (bpm)	77.4 ± 16.5	46.8 ± 17.8	N/A	—
Lowest blood pressure (mm Hg)	39.7 ± 8.3	48.0 ± 9.0	3.367	0.0008*
Highest blood pressure (mm Hg)	70.3 ± 15.3	71.5 ± 10.7	0.431	0.836
Time to diuresis (hours)	33.3 ± 20.7	24.3 ± 21.1	1.674	0.0478†

N/A = Not applicable.
*$p < 0.005$.
†$p < 0.05$.

Table V. Comparison of prenatal and delivery data (34 handicapped infants versus 34 control infants)

Factor	*Damaged*	*Controls*	χ^2	*p*
Premature labor	20	19	0.0	—
Premature rupture of membranes >24 hr	15	10	1.012	0.239
Antepartum hemorrhage or other	7	5	0.1012	1.192
Predelivery time in Unit <1 day	21	19	0.0607	1.571
Singleton	28	29	0.0	—
Twin	6	5	0.0	—
Vertex	14	21	2.119	0.095
Breech	17	10	2.211	0.089
All complications	8	5	0.38	0.535
Vaginal delivery	9	13	0.605	0.379
Cesarean section	25	21	0.605	0.379
Membranes intact	17	16	0.0	—
Fetal heart rate normal	22	23	0.0	—
Betamethasone therapy >36 hr	16	15	0.0	—
General anesthesia	25	18	2.277	0.085
Epidural anesthesia	5	13	3.7022	0.0326*
Male/female	15/19	18/16	0.235	0.732*
Small for gestational age	6	7	0.0	—

*$p < 0.05$.

minute Apgar scores in those who died, compared to the surviving normal control infants, and this was confirmed by equivalent lowering of mean cord venous pH and elevated base deficit. These infants also had a lower blood pressure recorded in the first 72 hours of life than did the control group. The highest blood pressure measured during this period was not different in the dead and control groups. In the dead group a longer time was required for urine output to exceed fluid intake (time to diuresis). They required plasma transfusion more often than the control group.

It was not considered suitable to compare ventilatory parameters in the dead versus control groups, since those who died tended to do so in the first few days and, therefore, were given assisted ventilation for a shorter period of time. In addition, higher inspired oxygen concentrations, ventilatory peak pressures, and ventilator rates probably reflect terminal care as well as poorer lung function, and it was not possible to assess the relative contribution of each of these factors.

Handicapped infants versus control infants. The results of analysis of prenatal and delivery factors in the handicapped infants compared to control infants is shown in Table V.

Table VI. Numerical neonatal factors in handicapped infants versus control infants (±SD)

Neonatal factor	*Handicapped*	*Control*	*T*	*p*
Mean gestation (wk)	28.2 ± 1.0	28.1 ± 1.2	0.227	0.91
Mean weight (gm)	1077.9 ± 207.9	1031.9 ± 183.5	0.968	0.83
Apgar score at 1 min	4.8 ± 2.2	5.0 ± 2.3	0.324	0.87
Apgar score at 5 min	7.6 ± 1.7	7.8 ± 1.5	0.754	0.77
Cord venous pH	7.31 ± 0.07 (n = 25)	7.33 ± 0.06 (n = 23)	0.85	0.798
Cord base deficit	5.6 ± 5.3	4.9 ± 2.9	0.555	0.794
Duration of assisted ventilation (days)	7.9 ± 5.8	5.6 ± 6.7	1.46	0.0728
Duration of oxygen therapy (days)	27.9 ± 17.2	31.8 ± 23.9	0.152	0.937
Maximum oxygen (mm Hg)	58.4 ± 19.9	45.8 ± 18.4	2.62	0.005*
Maximum pressure (cm H_2O)	21.2 ± 6.3	18.9 ± 6.4	1.396	0.082
Maximum rate (bpm)	50.4 ± 18.2	40.2 ± 22.7	1.975	0.025†
Lowest blood pressure (mm Hg)	44.7 ± 11.9	47.9 ± 10.4	0.69	0.75
Highest blood pressure (mm Hg)	68.3 ± 15.2	67.3 ± 11.7	—	—
Time to diuresis (hr)	20.7 ± 10.2	26.2 ± 15.8	1.57	0.059

*$p < 0.005$.
†$p < 0.05$.

Table VII. Other neonatal variables

Neonatal variable	*Dead*	*Control*	*T/χ^2*	*p*	*Handicapped*	*Control*	*T/χ^2*	*p*
Initial hemoglobin level	15.6 ± 2.5	15.5 ± 1.9	0.255	0.898	15.3 ± 1.9	16.2 ± 2.4	1.63	0.052
Assisted ventilation	36	37	0.0	—	33	34	0.0	—
Respiratory diagnosis								
Respiratory distress syndrome	26	16	4.18	0.024*	16	13	0.24	0.72
Wet lung syndrome	4	22	21.17	<0.0001†	15	21	1.476	0.16
Pneumothorax	14	10	0.54	0.41	5	8	0.38	0.54
Patent ductus arteriosus	8	21	N/A	N/A	19	17	0.059	1.59
Plasma transfusion	31	16	10.49	0.00065†	16	14	0.059	1.59
Subependymal hemorrhage	1	2	0.0	—	1	4	0.863	0.28
Intraventricular hemorrhage	21	6	11.10	0.00047†	16	4	8.571	0.0019†
Infection	28	19	3.43	0.039*	19	14	0.942	0.26

*$p < 0.05$.
†$p < 0.005$.

In the group of infants with handicaps detected at follow-up, there was a slight increase in the number who were found to be breech presentations ($p = 0.09$). This effect was not associated with vaginal delivery since, of the total of 27 infants in the handicapped group plus control infants who were breech presentations, all but four were delivered semielectively by cesarean section.

Epidural anesthesia was used more frequently in the control group than in those who were handicapped ($p < 0.05$).

There was a slight preponderance of female infants in the handicapped group compared to the control group (19 of 34 versus 16 of 34), but this was not significant.

In the handicapped group, the incidence of infants who were small for gestational age was not increased over that in the control group.

The condition at birth is shown in Table VI. When the infants who were handicapped were compared to the control infants, there were no significant differences found in condition at birth. All values for mean Apgar scores, cord venous pH, and base deficit appeared to show a trending toward the better in the control group, but none of the differences were significant. The duration of assisted ventilation was longer in the handicapped group and the maximum inspired oxygen concentration, peak ventilatory pressures, and highest ventilator rate were all increased in this group compared to the control group.

There were no differences in the lowest and highest recorded blood pressures (first 72 hours) or the need for plasma transfusion in the handicapped and control infants.

The time to diuresis (output exceeding input) in hours was longer in the control group than in those who were handicapped

Other neonatal factors. These results are shown in Table VII.

Dead versus control infants. There was no difference in the initial hemoglobin levels between the dead and control groups, and virtually all infants in both groups were treated with assisted ventilation since, in this unit, it is usual to provide this support to any infant weighing <1500 gm who demonstrates respiratory difficulty.

Respiratory distress syndrome, as defined in a previous report,[14] was diagnosed much more frequently in the dead group, whereas wet lung syndrome was found more often as a respiratory diagnosis in the control group. The incidence of pneumothorax was not different in the two groups.

Intraventricular hemorrhage of grade II and above was seen in a larger number of the dead infants than in the control infants, whereas there was no difference in the incidence of isolated subependymal hemorrhage (grade I).

Until mid-1980, the diagnosis of intracranial hemorrhage was on clinical and autopsy grounds; since that time routine real-time sector ultrasound scans have been performed on the fourth day of life and repeated as indicated.

Infection, defined as sepsis, meningitis, pneumonia, or necrotizing enterocolitis, proved or suspected by white blood cell counts of <5000/mm^2 or >30,000/mm^2 or a platelet count of below 100,000/mm^2, was seen more frequently in those who died than in the control group ($p < 0.05$).

It was not considered appropriate to compare the incidence and severity of patent ductus arteriosus in the dead and control groups, since many of the dead infants died too early to have developed signs and symptoms of this condition.

Handicapped infants versus control infants. There were no significant differences in initial hemoglobin level, need for assisted ventilation, type of respiratory disease diagnosed, incidence of patent ductus arteriosus, pneumothorax, or proved or suspected infection between the handicapped group and the control group.

The handicapped infants required a longer period of assisted ventilation, higher inspired oxygen concentrations, and higher pressures and rates than did the control infants, but there was no difference in the overall time in oxygen therapy.

There was also no significant difference in the highest or lowest blood pressure recorded between the handicapped and control groups. In the control group compared to the handicapped group, a slightly longer time was required to reach diuresis.

The incidence of intraventricular hemorrhage was again significantly associated with the handicapped group when compared with the control group ($p < 0.005$).

Comment

This study was undertaken in an attempt to delineate factors militating against an intact outcome in a group of high-risk infants subjected to a standardized obstetric and neonatal approach in an integrated perinatal facility. All high-risk pregnancy situations were managed according to the policies of the unit, and all newborn infants were subject to the same approach.[14]

In the interpretation of the data, it is understood that the conclusions of the study are specific to the population delivered at Women's College Hospital.

It would appear that, contrary to our traditional understanding, there is a difference between the factors leading to death of the newborn infant and those leading to damage in survivors at the same gestational age. The initial condition of the infant, as evidenced by Apgar scores, cord vein pH, and base deficit, appears to be correlated with neonatal death but does not seem to be related to subsequent handicaps. Prenatal factors, related to both pregnancy complications and maternal disease processes, seem to be relatively unimportant in ascertaining whether the infant will survive. The findings of significant association with death and time in the Perinatal Unit of <1 day, an abnormal fetal heart rate prior to delivery, and time of betamethasone administration of <36 hours may simply reflect that the fetal condition was assessed as severely compromised prior to the decision to deliver.

It has been demonstrated that, in the infant with respiratory distress, the time to diuresis is a measure of recovery; those with a shorter time to maximum diuresis have both an increased survival and a shorter time on assisted ventilation.[15] The finding of a significantly longer time to initiation of diuresis, defined in this study as the age in hours at which output exceeded intake, in the dead infants would tend to support this finding.

As might be anticipated, intraventricular hemorrhage was found more frequently in infants who died than in those who survived and were normal. Infection, either proved or suspected, was also seen more often in those who died compared to those who did not.

In this study, however, contrary to other reports, the occurrence of pneumothorax and patency of the ductus arteriosus was not seen significantly more often in those who died than in those who survived. These have been associated, in other reports, with the development of intracranial hemorrhage.[16, 17] This association was not seen in our study and, indeed, a separate analysis could not define perinatal factors associated with the occurrence of intracranial hemorrhage that were different from those in infants who did not suffer this complication. This seems to confirm a previous finding from this Center reported recently.[18]

In the comparison of handicapped infants with their control group, very few significant differences were found in this study, particularly in the predelivery period. Breech presentation occurred significantly more often in those handicapped versus the control group.

The finding of poorer, long-term outcome in infants who have been in a persistent breech position, irrespective of the mode of delivery, has been reported in the past and seems to hold even when infants with known abnormalities associated with neurological abnormalities are excluded, as in this study.[19, 20]

The finding of an increased use of epidural anesthesia in those surviving normally over those surviving with handicap may indicate that there was more time available to deliver the former, or it may indicate some adverse effect of general anesthesia in this group of very low–birth weight infants.

In the infants in this study, there was no evidence that the initial condition at birth, predictable from abnormalities in fetal heart rate patterns prior to delivery or as actually measured by Apgar scores, cord vein pH, or base deficit, was predictive of a normal outcome versus an abnormal one. This was in contrast to the findings in the group that died and is contrary to the findings of most in the field.

The infants who were handicapped did require a longer period of assisted ventilation, with higher pressures, rates, and inspired oxygen concentration, suggesting a worse acute course, although the time to diuresis was not greater than in the control infants and, indeed, appeared to be somewhat shorter. The handicapped infants did not develop pneumothoraces more often, however, and did not require a longer time of receiving oxygen, suggesting that chronic lung damage was no more frequent in those with subsequent handicap than in the normal control group. Elevation or lowering of blood pressure, the need for plasma transfusion, and the development of a significant patent ductus arteriosus were no different in the two groups. These findings all suggest that the circulatory homeostasis in the two groups was not significantly different.

Intraventricular hemorrhage, however, was found more frequently in those showing subsequent handicap, compared to the normal control infants, as it was in the dead group versus the control group. Inasmuch as no differences were demonstrated in this study between handicapped and control groups with respect to initial status at birth and severe complications in the neonatal period that might be expected to be associated with intraventricular hemorrhage, it is clear that this study fails to shed any light on the perinatal factors responsible for the development of this complication. Moreover, there were handicapped infants who did not have intraventricular or other intracranial hemorrhage and who did not differ in the perinatal factors from infants who had these hemorrhages.

The place of ischemic lesions in this particular series was not evaluated, but no differences in occurrences likely to be associated with hypoperfusion were detected in either the damaged or control groups. There may be episodes of cerebral hypoperfusion occurring prenatally that either are not evident by biochemical status at the time of delivery or are not reflected by the parameters examined in this study.

From this study, it would appear that the factors involved in neonatal death in this group of premature infants are not the same as those predisposing to handicap in survivors. The explanation may be that the degree to which adverse factors are operative is sufficient to demonstrate significance in the dead group, whereas these same factors in the handicapped group exert their influence at a less acute level, insufficient to be demonstrated with the numbers included in the study group.

Another explanation may be that, indeed, there are different factors involved in damage and that these either are neonatal in origin and have not been demonstrated by the current analysis or are prenatal in origin and undetected by the usual methods of antenatal surveillance or by the available assessments of initial condition at birth. The latter explanation would suggest, along with the finding of increased morbidity associated with breech position, that there is a group of fetuses who have suffered cerebral damage in utero that is not presently detectable either before or at birth.

There is an obvious need for more refined neonatal assessment tools and also for innovations in antenatal diagnosis to attempt to answer these important questions.

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A prospective study of microbial infection in stillbirths and early neonatal death

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No morphologic cause of death was found in 67.8% of 33 perinatal deaths. The mothers had experienced a previous loss in 48% of cases. Inflammation occurred in 65.6% of the cases of perinatal death compared to 4% of a control group ($p < 0.001$) and in 73.1% of organism-positive cases of perinatal death compared to 7.1% of organism-positive cases of the control group ($p < 0.001$). Incidence of maternal fever or prolonged membrane rupture was not statistically significant. Bacteria were present in 33.3% of the cases of perinatal death (not significant), with more pathogenic strains occurring in this group ($p = 0.0028$); 75.0% had inflammation compared to 0% of the control group (not significant). Genital mycoplasmas were detected in 78.8% of cases of perinatal death compared to 32.3% of control cases ($p < 0.001$). Positive cultures ($p = 0.0142$) and elevated antibody titers in the fetus or neonate ($p = 0.00052$) or in the mother ($p = 0.0122$) occurred significantly more often than in control cases. Inflammation occurred in 78.9% of mycoplasma cases ($p = 0.00032$); incidences of maternal fever and prolonged membrane rupture were not significantly different. In perinatal death cases 20% had evidence of viruses, and 3.2% had evidence of chlamydia. Evidence of mixed microorganisms occurred in 46% of cases of perinatal death. However, 78.6% (11 of 14) with only one organism had *Ureaplasma urealyticum* (33.3% overall). Of the Ureaplasma-positive cases, 72.7% had inflammation, 45.5% had fever, and only 18.2% had prolonged membrane rupture compared to 28.6%, 0%, and 16.6%, respectively, in a negative-microorganism group with perinatal deaths. Our observations strongly support the concept that infection is a major cause of perinatal death and that genital mycoplasmas play a significant role. (AM J OBSTET GYNECOL 1985;151:238-49.)

Key words: Microbial infection, mycoplasmas, stillbirths, early neonatal death

Despite significant advances in maternal and obstetric care which reduced stillbirths and early neonatal deaths between 1972 to 1981, the incidence is still high. In Ontario there were 1534 stillbirths in 1972 and 874 stillbirths and 689 neonatal deaths up to 20 days of age in 1981.[1] However, 56.9% of the latter were under 1 day of age, accounting for 2.5% of all deaths. In Canada there were 2622 stillbirths and 2470 neonatal deaths in 1980.[2] The situation is much worse in Third World countries where poverty and poor nutrition increase

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the risk of perinatal death thought to be due to the high rates of infection.[3] In addition, except for cases with severe congenital abnormalities or maternal disease (e.g., diabetes mellitus), a satisfactory explanation for many stillbirths and early neonatal deaths is not found. If one examines all causes of death listed for stillbirths in Ontario in 1981, only 29 of 80 causes cited (35%) can truly be considered lethal or even possibly lethal. In only 0.8% of cases (7 of 874) was infection (mother or fetus) attributed as the cause of death. In addition, in 12.6% of cases (110 of 874) no probable cause of death was listed.[1] In research studies 16% of perinatal deaths have been associated with infectious etiologies[4] at a time when mycoplasma and chlamydia were not considered.

Studies undertaken in the past several years have shown a significantly higher rate of spontaneous abortion, stillbirth, prematurity, and perinatal morbidity and mortality among mothers with positive placental cultures for *Ureaplasma urealyticum* or *Mycoplasma hominis*.[5-10] Based on culture and histologic evidence of inflammation, studies show that stillborn infants and neonates can be infected with *U. urealyticum*[5, 8] (also unpublished observations). As early as 22 weeks of gestation, stillborn infants may have a significantly higher antibody response to *U. urealyticum* than their mothers.[12] A higher mortality rate was also found among neonates in our intensive care, with occurrence of respiratory disease and elevated antibody responses to *U. urealyticum.*[11]

Although large-scale studies have reported on the correlation between the presence of genital mycoplasma in the placenta and pregnancy loss,[6, 7] the outcome as reflected by both mother and fetus has not been assessed. To determine whether there was an association between infection and perinatal death, we examined 33 cases of pregnancy loss and 31 normal deliveries. These cases were assessed for the presence of inflammation by histologic examination of the placenta, lung, and most other organs; by the isolation of microorganisms (bacteria, mycoplasmas, chlamydia and viruses); by the measurement of antibodies to *U. urealyticum* serotypes, *M. hominis,* chlamydia, and viruses; and by a clinical history of fever or prolonged membrane rupture. We undertook this study in an effort to determine whether microbial infection played a role in stillbirths and early neonatal deaths. This concept is basically accepted but rarely stated as the cause of death.[12]

Material and methods

Population. Control and study subjects were patients admitted to Women's College Hospital or Toronto General Hospital for delivery during 1982 and 1983. All cases which met the criteria and for which consent forms for autopsy were obtained (except weekend autopsies) were studied. All patients were covered by a provincial health insurance plan. Some patients were referred to the Regional High Risk Perinatal Unit at Women's College Hospital just prior to delivery; thus complete clinical histories were not available in all cases, and many pregnancies were managed by obstetricians outside our institutions.

The 33 perinatal deaths consisted of 28 fetuses, stillborn between 20 to 42 weeks of gestation, and five neonates who died within 48 hours of delivery. They were examined at autopsy to determine the cause of death. In a few cases the fetuses weighed <500 gm although they were considered to be of 20 weeks' gestation. For some analyses the perinatal deaths were classified as follows: (1) group 1 consisted of cases with a known cause of death, including multiple lethal congenital abnormalities, severe hydrops fetalis with acute polyhydramnios, and cases with a questionable cause of death such as multiple congenital abnormalities and manageable defects complicated by extreme prematurity; (2) group 2 consisted of cases with no morphologic or anatomic cause of death.

The control group consisted of 31 random cases of normal term deliveries between Sunday night and Friday noon (to accommodate laboratory facilities). The criteria for a normal delivery consisted of a term delivery with membranes ruptured (<24 hours). The microbiology portion of the study was complete in all 31 cases, and histology was done on the placenta in 25 cases. All clinical histories available were reviewed to determine whether there was an elevation in maternal temperature (≥37.5° C) or prolonged membrane rupture in the mother (≥24 hours).

Specimen collection. In the control group, maternal and umbilical cord bloods were collected at delivery. A 2 cm cube of placenta was taken in the delivery suite with aseptic precautions and placed in a sterile container. All samples were refrigerated and taken to the microbiology laboratory as soon as possible (4 to 12 hours for night deliveries) and set up immediately. The placenta was sent to the Department of Pathology for histologic examination. In the cases of perinatal death, the placenta and fetus or neonate were submitted to the pathology department for histology or autopsy; maternal serum was also requested. A blood sample drawn from the fetus, placental tissue, and postmortem specimens were submitted for microbiology studies at autopsy. If there was an extended period between fetal death and delivery or if the fetus was of very low birth weight, occasionally the blood could not be obtained, was hemolyzed, or the tissues were autolyzed.

Autopsy procedure. A detailed autopsy was performed in all cases according to a specific protocol within 24 to 48 hours after delivery or death. Specimens

Table I. Summation of obstetric history of study patients

	No. of patients	Mean maternal age (yr)	Mean gestational age (wk)	Weeks of gestation (%)			
				18-27	*28-31*	*32-37*	*≥38*
Perinatal death group	33	28.1 (18-37)	27.4 (18-40)	54.8	16.1	19.4	9.7
Control group	31	28.3 (17-40)	39.6 (38-42)	0	0	0	100

for cultures of the lungs and liver were taken at the beginning of the autopsy with the use of "flamed" forceps and a sterile blade. By means of a sterile syringe, blood for serology was drawn after heat-sterilizing of the surface of the inferior vena cava. Tissue for histologic sections was taken from the placenta, lungs, liver, heart, and kidneys. Sections from the thymus, spleen, and brain were also taken when possible. Particular emphasis was placed on tissue from the lungs, with sections being taken from each lobe. Five blocks were taken from each placenta, and these included one strip of free membranes and umbilical cord, and four full-thickness blocks of the placental cake. Histologic sections were stained with hematoxylin and eosin, and when evidence of inflammation was seen, sections were stained for bacteria (Gram and Gomori methenamine silver stains).

The histologic criterion used for acute inflammation of the placenta (chorioamnionitis) was the presence of accumulations of polymorphonuclear leukocytes in the amnion and chorion on the fetal surface and in the free membranes. Polymorphonuclear leukocytes were generally seen in the chorionic plate, under the amnion, and at times in the intervillous space under the chorionic plate.[12] The histologic criteria for acute inflammation in the lungs (pneumonitis) included the presence of polymorphonuclear leukocytes and fibrin in the alveolar spaces. All histologic sections of the lungs and the placenta were examined blind by one of us (J. B.). In only a few cases, autolysis of the fetus prevented valid conclusions.

Microbiology. The placental tissue was surface-sterilized by burning and cut open, and a block of tissue for cultures cut from the inside to avoid vaginal contamination. The placenta, lung, and liver tissue were minced lightly and inoculated on media to isolate aerobic and anaerobic bacteria and fungus. A Gram stain was made of each specimen. Cultures for aerobic bacteria and fungus were inoculated on blood agar, bile salt plates, Levinthal medium, and Sabouraud agar. For anaerobes, specimens were planted on blood agar for anaerobic incubation and into a Robertson chopped meat broth. For the last 12 cases, a Spirrow medium was also included for *Campylobacter jejuni*, which was incubated at 42° C for 48 hours in 7% carbon dioxide.

The tissue specimens were lightly minced, not ground, and inoculated into two broth and agar culture systems for the isolation of large-colony mycoplasmas and *Ureaplasma urealyticum*.[9] Three 10-fold serial dilutions were made for broth cultures. Tissues were frozen at −70° C in transport media containing gentamicin (10 μg/ml), streptomycin (50 μg/ml), and nystatin (25 μg/ml) for the isolation of chlamydia and cycloheximide-treated HeLa cells.[13] The tissues were frozen at −70° C and set up for the isolation of viruses in skin fibroblasts, green monkey kidney, and HeLa 293 cells.[13]

Serology. The metabolic inhibition test, which we modified to increase sensitivity,[10] was used to measure the patients' antibody response to the original eight serotypes of *U. urealyticum* and *M. hominis* (PG-21). Chlamydial antibody titers were detected by the microimmunofluorescence test.[14] The hemagglutination test was used for rubella, complement fixation test for cytomegalovirus and herpes simplex virus, and the neutralization test for enterovirus and Coxsackie B virus. For mycoplasma, chlamydia, and viruses, an antibody titer of $\geq 1:64$ was scored as positive. Rubella positive titers were excluded, since most women were immunized.

Statistical methods. Comparisons were made between the group with perinatal deaths and the control group and between groups 1 and 2 within the perinatal death group. In each test of significance, either the χ^2 test, corrected for continuity, or Fisher's exact test was used. Results have been considered significant if $p < 0.05$ in a two-tailed test and of "borderline significance" if $p < 0.10$ in a two-tailed test. The size of the association has been calculated for all significant results with the use of the odds ratio modified by Woolf for small frequencies.[15] The 95% confidence interval for this quantity has also been given. Because the numbers of cases are small in many of the comparisons, the confidence interval may be wide, indicating that our estimates of the relative risk are imprecise.

Results

Characteristics of the populations studied. Frequently in a search for the cause of death in perinatal losses no specific cause can be found. In this study a specific morphologic cause was found in only five cases

Table II. Evidence of infection in perinatal death compared to normal deliveries

	Inflammation (%) *(n = 32, 25*)*	*Fever* *(n = 33, 26*)*	*Prolonged membrane rupture* *(n = 32, 31*)*	*Culture and serology positive* *(n = 33, 31*)*
Perinatal death group	65.6†	30.3	29.0	78.8
Group 1	44.4	0.0	22.2	40.0
Group 2	77.3	39.1‡	31.8	95.6§
Control group	4.0	23.1	0	64.5

*Number of cases are given for perinatal death and control groups respectively.
†Comparison of perinatal death group to control group; $p < 0.001$.
‡Comparison of group 2 to group 1; $p = 0.025$.
§Comparison of group 2 to group 1; $p < 0.001$.

and a possibly lethal problem in five, a total of 32.2%, which was identified as group 1. Thus in 67.8% of these cases no morphologic cause of death was found, and these were identified as group 2.

A comparison of the obstetric histories of the group with perinatal deaths and the normal-delivery control group appears in Table I. The mean maternal ages and age ranges were comparable. However, there was a history of previous pregnancy loss in 48% of women, with current perinatal loss compared to 3.2% in the 31 normal deliveries ($p < 0.001$). This incidence of previous losses in control subjects is below average and may occur because patients with previous late losses would be managed through the High Risk Service and not be considered as normal. Among 28.0% of cases, this loss was a first pregnancy with no prior history to indicate high risk. In 24% of women with previous pregnancies (one to three) this was their first pregnancy loss. In the group with perinatal deaths, 54.8% of pregnancies were lost at <28 weeks gestation, and 29.0% were lost after 32 weeks' gestation, when a reasonable chance of survival should have existed.

Pregnancy outcome associated with evidence of infection. A diagnosis of infection can be made on the basis of two or more of the following criteria: the presence of fever, detection of microorganisms by culture and serology, or histologic evidence of inflammation. The presence of these factors is examined in Table II for cases with valid histology and records of fever and prolonged membrane rupture. Among the group with perinatal deaths, 65.6% had evidence of inflammation, which was significantly higher than the 4.0% in the control group ($p < 0.001$). The relative odds of a perinatal death case having inflammation was 30.5 times higher than for a control case, with 95% confidence limits of 5.1 to 184. The difference between groups 1 and 2 was too small to be statistically significant ($p = 0.1004$, two-tailed test). On the other hand, this observation suggests that infection may play a role in group 1 cases in conjunction with possible morphologic causes of death.

When cases were evaluated for the presence of maternal fever, which is indicative of infection, there was no significant difference between the perinatal death and control groups. In fact, fever occurred in four control cases with no evidence of inflammation in the placenta or lung and no microorganisms isolated. However, within the perinatal death group, group 2 exhibited significantly elevated fever in 39.1% of cases compared to 0% in group 1 ($p = 0.025$). Although an elevated temperature must be considered significant, overall it is not a reliable indicator of a poor outcome resulting from infection. The role of maternal fever will be clarified as each group of microorganisms is discussed.

Prolonged membrane rupture prior to delivery has been considered a major factor leading to infection in pregnancy. However, in this study only 29.0% of the perinatal death cases had prolonged membrane rupture. It is not valid to compare this percent to the control group, since the occurrence of prolonged membrane rupture was an exclusion criterion for the control group. When we compare group 2 to group 1, we find no apparent difference, which also supports the concept that prolonged membrane rupture seems not to be the main contributing factor to an infectious cause of death.

Evidence of microorganisms was found by isolation or serology in 78.8% of perinatal death cases compared to 64.5% of the control group. The overall incidence was higher than expected in the control cases because evidence of bacteria was found in six controls, mycoplasmas in ten, chlamydia in five, and virus antibodies in eight. In only one of these cases was there evidence of inflammation. However, when we compared the two perinatal death groups, a significantly higher incidence of microorganisms occurred in group 2 compared to group 1 ($p = 0.00115$, Fisher's exact test) with an odds ratio of 21.67 (limits, 2.8 to 167). Thus evidence of infection was more common in the group with perinatal deaths. The organisms present did not indicate merely opportunistic infections, affecting group 1 pregnancies

Table III. Correlation between the detection of microorganisms and evidence of infection

	% of positive cases		
Cases positive by culture and/or serology	*With inflammation*	*With fever*	*With prolonged membrane rupture*
Culture and serology			
Perinatal death group	73.1* (19/26)	42.3 (11/26)	30.8 (8/26)
Control group	7.1 (1/14)	14.3 (2/14)	(0)
Culture			
Perinatal death group	84.2† (16/19)	45.0 (9/20)	30.0 (6/20)
Control group	0.0 (0/7)	12.5 (1/8)	(0)
Serology			
Perinatal death group	68.0 (17/25)	34.6 (9/26)	26.9 (7/26)
Control group	10.0 (1/10)	11.1 (1/9)	(0)

*Comparison of perinatal death group to control group; $p < 0.001$.

†Comparison of perinatal death group to control group; $p = 0.00036$.

already in difficulty for other reasons, since organisms and inflammation were more common in group 2 cases, in which no clear-cut morphologic causes of death were found.

Correlation between the detection of microorganisms and evidence of infection. Since the detection of inflammation was significant in the group with perinatal deaths and since 58.1% of control cases had evidence of microorganisms, we assessed the correlation between the presence of microorganisms with inflammation. In patients shown positive for any microorganism by culture and/or serology, 73.1% of the group with perinatal deaths had histologic evidence of inflammation in the placenta or fetal lung compared to 7.1% of control cases ($p < 0.001$), as shown in Table III. The odds of microorganism-positive patients with perinatal deaths having inflammation are 23.4 times higher than in similar control patients (limits, 3.6 to 154). If one examines cases in which cultures are positive for bacteria, mycoplasma, chlamydia, or viruses, a very significant correlation exists, with the presence of inflammation found in 84.2% of perinatal death cases compared to 0% of control cases ($p = 0.00036$). The odds ratio increases dramatically to 165, indicating a very strong association between perinatal death and inflammation in culture-positive cases. If serologic evidence of infection (mycoplasma, virus, or chlamydia) is assessed, again there is a significant correlation, with inflammation present in 68.0% of perinatal death cases compared to 10.0% of control cases ($p < 0.015$) with an odds ratio of 13.0 (limits, 1.9 to 87.7). Thus a much higher incidence of inflammation occurred in the perinatal death group compared to that in the control group in cases with organisms detected by culture, serology, or both methods.

The correlation between maternal fever prior to delivery and the presence of organisms was difficult to assess because of the high incidence of fever with no evidence of inflammation among control subjects. Perhaps a higher baseline temperature should be considered in future studies. Overall there was a higher percentage of fever in organism-positive cases, but this was not significant. We also observed approximately a 30% incidence of prolonged membrane rupture in perinatal death cases that were positive by culture, serology, or both.

Since microorganisms may be detected which reflect past infection or nonpathogenic strains, we compared the presence or absence of microorganisms with factors associated with infections. Among perinatal death cases with positive culture for organisms, 19 of 25 (76.0%) had inflammation, 10 of 26 (38.5%) had fever, and 8 of 25 (32.0%) had prolonged membrane rupture, compared to 2 of 7 (28.6%), 0 of 7 (0%) and 1 of 6 (16.7%) in culture-negative cases with perinatal deaths. Although not reaching statistical significance due to small numbers, these observations also suggest an association between organisms found in perinatal death cases and evidence of infection.

Types of microbial infection found in perinatal deaths. For the detection of microbial infection, all isolates and antibody titers to genital mycoplasmas, virus, and chlamydia ($\geq 1:64$) were scored as positive. We have chosen to report all organisms detected at this time because such comprehensive microbiology studies are new and the pathogenic role of organisms may change. No significant difference was observed in the incidences of bacteria, chlamydia, and viruses between the group with perinatal deaths and the control group (Table IV). In fact, there was a higher prevalence of chlamydia and viruses resulting from elevated antibody titers in the control group than in the group with perinatal deaths. There were no significant differences for these organisms between groups 1 and 2.

However, a significantly higher incidence, 78.8% of the perinatal death cases, had evidence of genital mycoplasmas compared to the 32.3% of control cases ($p < 0.001$). The odds ratio for mycoplasma-positive cases being in the group with perinatal deaths was 7.23 (limits, 2.4 to 21.6). There were also more mycoplasma-

Table IV. Types of microorganisms detected in perinatal deaths compared to those found in normal deliveries*

	Bacteria	*Mycoplasma*	*Chlamydia*	*Virus*
Perinatal death group	33.3 (11/33)	78.8† (26/33)	3.2 (1/31)	20.0 (4/20)
Group 1	20.0 (2/10)	50.0 (5/10)	11.1 (1/9)	14.3 (1/7)
Group 2	39.1 (9/23)	91.3‡ (21/23)	0.0 (0/22)	23.1 (3/13)
Control group	19.4 (6/31)	32.3 (10/31)	16.1 (5/31)	26.6 (8/30)

*Positive by culture or serology.

†Comparison of perinatal death group to control group; $p < 0.001$.

‡Comparison of group 2 to group 1; $p = 0.0322$.

positive cases in group 2 compared to group 1 ($p = 0.0322$) with an odds ratio of 8.60 (limits, 1.47 to 15.87).

Evidence of infection in bacteria-positive cases. Although there was no difference in the isolation rates for all bacteria, pathogenic strains were found significantly more often in the group with perinatal deaths (27.3%) than in the control group (0.0%) ($p = 0.0028$). The odds ratio for pathogenic bacteria being found in the perinatal death group was 24.4 (limits, 1.4 to 440). The species of bacteria isolated are listed in Table V. Although all specimens were screened for unsuspected bacteria by Gram stain, no cases were observed in which there was a positive Gram strain and no appropriate organism isolated. In most cases, growth of bacteria was scanty, so the missing of isolates resulting from overgrowth was not likely. Despite use of staining and suitable culture methods, no cases of unusual bacteria such as *Listeria* or Campylobacter sp. were detected.

Aerobes were isolated from 36.4% of the group with perinatal deaths compared to 16.1% of the control group ($p = 0.05$) with an odds ratio of 5.24 (limits, 1.55 to 17.7). There was no difference in the isolation of anaerobes, probably the result of small numbers, but all the anaerobes in the perinatal death group were from group 2.

In bacteria-positive cases (Table VI), 75.0% of the perinatal death group and 80.0% of group 2 had evidence of inflammation in the placenta or lung compared to 0% in the control group. Because of the small number of cases this is not significant. There was no significant difference in the incidence of maternal fever in the bacteria-positive cases, mainly because of the high incidence of fever of unknown origin in the control cases. Prolonged membrane rupture occurred in 36.4% of the bacteria-positive cases. When we compared group 2 to group 1, the group 2 bacteria cases were associated with a higher incidence of inflammation, fever, and prolonged membrane rupture than were group 2 cases but were not yet significant because of the limited number of cases.

Evidence of infection in mycoplasma-positive cases. The isolation of *U. urealyticum* and *M. hominis* was examined in all groups. Mycoplasmas were isolated significantly more often from perinatal death cases (39.4%) than from control cases (9.7%) ($p = 0.0142$) as shown in Table VII. The isolation of mycoplasmas in 56.5% of group 2 was significantly higher than the

Table V. Bacterial species isolated

No. of cases	*Aerobes*	*Anaerobes*
Perinatal death group		
12	*Klebsiella aerogenes** *Streptococcus faecalis*	
13	*Staphylococcus epidermidis* *Acinetobacter anitratus*	
16	*S. epidermidis*	*Bacteroides melaninogenicus**
21	*Citrobacter freundii**	
22	β-Hemolytic streptococcus* Lancefield group B streptococcus	
25	*Pseudomonas aeruginosa**	*B. bivius*
26	*P. aeruginosa**	*B. melaninogenicus**
27	*S. epidermidis* *S. faecalis*	*B. bivius* *B. fragilis**
30	β-Hemolytic streptococcus Lancefield group B streptococcus *S. aureus**	
31	*K. pneumoniae**	
32	*S. epidermidis* *S. faecalis* *C. albicans*	
34	*S. faecalis*	
Control group		
5	Diphtheroids	
9	*S. epidermidis*	
18	*S. epidermidis* *S. faecalis*	
19	*S. viridans*	
22	*S. viridans*	
28		*B. vulgatus*

*Indicates more pathogenic strains.

Table VI. Association of inflammation, fever, and prolonged membrane rupture in bacteria-positive cases

			*Bacteria-positive cases (%)**		
	Aerobes (%)	*Anaerobes (%)*	*Inflammation*	*Fever*	*Prolonged membrane rupture*
Perinatal death group	36.4* (12/33)	17.4 (4/23)	75.0 (9/12)	33.3 (4/12)	36.4 (4/11)
Group 1	20.0 (2/10)	0.0 (0/9)	50.0 (1/2)	0.0 (0/2)	0.0 (0/2)
Group 2	43.5 (10/23)	28.6 (4/14)	80.0 (8/10)	40.0 (4/10)	44.4 (4/9)
Control group	16.1 (5/31)	3.2 (1/31)	0.0 (0/4)	20.0 (1/5)	(0)

*Scored for cases positive for aerobes or anaerobes.

Table VII. Association of inflammation, fever, and prolonged membrane rupture in mycoplasma-positive cases

		Serology-positive cases (%)		*Mycoplasma-positive cases (%)**		
	Culture-positive cases (%)	$I \geq 1:64$†	$M \geq 1:64$†	*Inflammation*	*Fever*	*Prolonged membrane rupture*
Perinatal death group	39.4‡ (13/33)	45.8§ (11/24)	26.3‖ (5/19)	78.9¶ (15/19)	40.0 (8/20)	25.0 (5/20)
Group 1	0.0 (0/0)	14.3 (1/7)	0.0 (0/3)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
Group 2	56.5# (13/23)	58.8 (10/17)	31.3 (5/16)	83.3 (15/18)	42.1 (8/19)	26.3 (5/19)
Control group	9.7 (3/31)	0.0 (0/30)	0.0 (0/30)	11.1 (1/9)	12.5 (1/8)	(0.0) (0/10)

*Scored for cases positive by culture or serology.
†I, Infant; M, mother.
‡Comparison of perinatal death group to control group; $p = 0.0142$.
§Comparison of perinatal death group to control group; $p = 0.000052$.
‖Comparison of perinatal death group to control group; $p = 0.0122$.
¶Comparison of perinatal death group to control group; $p < 0.00032$.
#Comparison of group 2 to group 1; $p = 0.004$.

0% in group 1 ($p = 0.004$). The odds ratio of mycoplasma-positive cases being in the perinatal death group rather than in the control group was 5.4 (limits, 1.5 to 19.8). The odds ratio for a case with mycoplasma-positive isolation being in group 2 compared to group 1 was 27 (limits, 1.3 to 469). Thus the isolation of genital mycoplasmas from placentas and fetal/neonatal lungs was strongly associated with perinatal death.

The ureaplasma antibody response in the group with perinatal deaths was compared to the control group to determine whether the fetus/neonate made antibody in utero and to determine whether titers would be elevated compared to a control group. The fetus/neonate in 45.8% of the perinatal death cases had elevated antibody titers to at least one serotype of *U. urealyticum* compared to 0% of the control group ($p = 0.000052$) with an odds ratio of 52.0 (limits, 2.9 to 946). Of particular interest was the fact that only 14.8% of group 1 fetus-neonates exhibited elevated titers to *U. urealyticum* compared to 58.8% of group 2, but this was not statistically different ($p = 0.1190$). Among the mothers, 26.3% of perinatal death cases compared to 0% of control cases had elevated titers at delivery ($p = 0.0122$) with an odds ratio of 23.14 (limits, 5.1 to 105). Among the group 2 mothers, 31.3% had elevated titers compared to 0% of group 1 mothers. Antibody response to *U. urealyticum* in the fetus and their mothers correlated significantly with being in the perinatal death group and especially with being in group 2 rather than in group 1.

When we examined the association of mycoplasmas to the presence of other factors indicative of infection (Table VII), inflammation in mycoplasma-positive cases was significantly more common in the perinatal death group (78.9%) than in the control group (11.1%) ($p < 0.00032$). The odds of inflammation occurring were 19.5 times higher in cases with perinatal deaths than in the control cases (limits, 2.6 to 148). Within the perinatal death group, inflammation was found in 83.3% of group 2, compared to 0% in group 1. Therefore among cases with evidence of mycoplasma infection, outcome was correlated with the presence of an

Table VIII. Detection of chlamydia and viruses in perinatal deaths compared to those in normal deliveries

			*Positive cases**		
	Culture-positive	*Serology* $\geq 1:64$	*Inflammation*	*Fever*	*Prolonged membrane rupture*
Viruses					
Perinatal death group	2/19	2/13	4/4	2/4	3/4
Control group	ND†	8/30	0/7	0/8	(0)
Chlamydia					
Perinatal death group	0/22	1/24	0/1	0/1	0/1
Control group	1/31	4/30	0/3	0/3	(0)

*Scored for cases positive by culture or serology.
†ND, Not detected.

Table IX. Combination of organisms detected by culture or serology*

	Cases positive for			
	Bacteria	*Mycoplasmas*	*Chlamydia*	*Viruses*
Perinatal death group	n = 12 5 myco. C^+ S^+ 3 myco. S^+ 1 myco. S^+ chlam. S^+ 1 myco. S^+ virus C^+ S^+ 2 with no other organisms	n = 20 7 bact. C^+ 1 virus C^+S^+ 1 virus S^+ 11 with no other organisms	n = 1 1 bact. C^+	n = 4 1 bact. C^+ myco. S^+ 1 myco. S^+ 1 myco. C^+ 1 with no other organisms
Control group	n = 6 4 myco. S^+ 1 chlam. S^+ 1 with no other organisms	n = 24 4 bact. C^+ 1 chlam. C^+ 1 chlam. S^+ 1 bact. C^+ chlam. S^+ 17 with no other organisms	n = 5 1 bact. C^+ 1 myco. C^+ chlam. S^+ 1 myco. C^+ 1 myco. S^+ 1 with no other organisms	n = 8 1 bact. C^+ 1 myco. C^+ chlam. S^+ 2 myco. S^+ 4 with no other organisms

*Bact., Bacteria; myco., mycoplasma; chlam., chlamydia; C, culture; S, serology.

inflammatory response. Among 10 cases only positive for mycoplasma, 40.0% of the mothers with perinatal deaths had fever compared to 12.5% of control subjects and 25.0% had prolonged membrane rupture compared to 0.0%. There are not yet sufficient cases to make a statistical comparison. Fever was more common in mothers in perinatal death group group 2 than in control or group 1, although the numbers were too small for these differences to achieve statistical significance.

Evidence of infection in chlamydia and virus positive cases. Viruses and chlamydia have been associated with infection in pregnancy. In this study evidence of viral infections was observed in four cases of the perinatal death group (Table VIII). Cytomegalovirus was isolated from one neonate of a triplet gestation, with the two fatal cases (neonates 2 and 3) having a titer of 1:32. Membranes were ruptured for ≥24 hours. There was evidence of inflammation in the placenta and lung of neonate 2 but not in neonate 3 which had hyaline membrane disease. No other microorganisms were detected. Herpes simplex virus was isolated from one case with a titer of ≥1:64 and bacteria (*Bacteroides fragilis, B. bivius, Staphylococcus epidermidis,* and enteric streptococci) were isolated from the fetal lung. There was a history of fever, prolonged membrane rupture of 4 days' duration, and inflammation in the placenta but not in the lung. In one case an elevated titer to herpes simplex was detected along with an elevated titer to cytomegalovirus, and *U. urealyticum* was isolated from the fetal lung. Elevated titers to *U. urealyticum* were found in the other case with herpes simplex antibodies and multiple congenital anomalies. In the latter cases one neonate had elevated antibodies to *U. urealyticum* with multiple congenital anomalies. The numbers are too small to assess, but 4 of 4 virus positive cases had inflammation, 2 of 4 had maternal fever, and 3 of 4 had prolonged membrane rupture.

In this study, chlamydia were isolated from 0 of 22 perinatal death cases and 1 of 31 control cases. Elevated chlamydial antibody responses were found in 1 of 24 perinatal death cases and 4 of 30 control cases. In the perinatal death case a term infant had died of intrauterine asphyxia. Chlamydia was not isolated from placental tissue because of toxicity in the HeLa cells. Antibody titers were 1:256 in the mother and 1:128 in

Table X. Summary of results

Category	*Significance*	*Odds ratio*
Presence of inflammation in perinatal death group vs. control group	<0.001	30.5
Detection of any microorganisms		
Group 2 vs. Group 1	0.00115	21.7
Inflammation in perinatal death group vs. control group		
Culture-positive cases	0.00036	165
Serology-positive cases	<0.01	13.0
Any positive cases	<0.001	23.4
Detection of specific microorganisms		
Bacteria (pathogenic)	0.0322	8.6
Genital mycoplasmas	<0.001	7.2
Mycoplasma-positive cultures		
Perinatal death group vs. control group	0.0142	5.4
Group 2 vs. Group 1	0.004	24.4
Ureaplasma urealyticum–positive serology		
Infants: perinatal death group vs. control group	0.000052	52.0
Mothers: perinatal death group vs. control group	0.0122	23.1
Inflammation in mycoplasma cases		
Perinatal death group vs. control group	0.00032	19.5

the stillborn infant. There was a light growth of bacteria from placenta and lung but no evidence of inflammation, fever, or prolonged membrane rupture. These observations suggest that chlamydia infection may not have been involved in the fetal death. Elevated antibody titers in mother and infant were found in four control cases, but there was no evidence of inflammation or history of fever or prolonged membrane rupture.

Mixed infections in perinatal death. It soon became apparent that in the cases we studied, more than one microorganism was frequently detected (Table IX). Among the perinatal death cases, evidence of other microorganisms occurred in 10 of 12 bacteria-positive cases, 9 of 20 mycoplasma-positive cases, 1 of 1 chlamydia-positive cases, and 3 of 4 virus-positive cases. Thus in only two cases did bacteria alone play a role and in only one case was cytomegalovirus involved. Among the mycoplasma-positive cases, 55% had only mycoplasmas. This group accounted for 33.3% of the entire perinatal death group. When we compared the mycoplasma-positive perinatal death cases to perinatal death cases with no organisms detected, 72.7% (8 of 11) had inflammation compared to 28.6% (2 of 7) of the negative control cases, 45.5% (5 of 11) had fever compared to 0% (0 of 7) of control cases, and only 18.2% (2 of 11) had prolonged membrane rupture compared to 16.6% (1 of 6) of the control cases. The presence of inflammation and fever along with the significant elevation in fetal *U. urealyticum* antibodies strongly support the concept that genital mycoplasmas alone can cause infection in pregnancy leading to perinatal death.

Summary of results. Many factors can be examined in trying to understand the role of infection as a cause of perinatal death. The factors observed to have significance based on p values and odds ratios are summarized in Table X to facilitate this examination.

Comment

Perinatal death is still a major cause of pregnancy loss despite advances in obstetric and perinatal medicine. Frequently when autopsies are performed on such fetuses or neonates, no cause of death can be determined.[1] In other studies, an infectious cause of death has been documented in 22% of neonatal deaths and 6% of stillbirths.[4] However, there is increasing evidence that in addition to bacteria and viruses,[4] mycoplasmas[5-8] are associated with a high incidence of infection in pregnancy leading to pregnancy loss.

In this study, investigations were undertaken to determine the role of infection as a cause of stillbirth and early neonatal death. Evidence of inflammation, found by histologic examination of fetal organs and placenta, independent of culture results, were observed in 65.5% of cases. If cases within the perinatal death group which have no other cause of death are considered, inflammation was found in 77.3% of cases. Presence of inflammation correlated strongly with the presence of microorganisms by culture ($p = 0.00036$), by serology ($p < 0.01$), or by both methods ($p < 0.001$). The odds ratio of inflammation in culture-positive perinatal death cases was 165 times that in culture-positive control cases. The correlation of inflammation to serology was largely associated with elevated antibody titers to *U. urealyticum*. Thus in cases with demonstrated presence of microorganisms, inflammation was strongly associated with perinatal death, and particularly in group 2 where 95.6% cases had evidence of microorganisms. The presence of fever prior to delivery was not a good indicator that women were at risk of pregnancy loss. However, within the perinatal death group fever occurred significantly more often in group 2 than in group 1. Prolonged membrane rupture occurred in only 30% of cases and was slightly higher in group 2 (31.8%) than in group 1 (22.2%). Thus it is not the only factor leading to intrauterine infection, which is supported by the fact that mycoplasmas have been isolated from amniotic fluid without evidence of ruptured membranes. Evidence of infection is therefore more common in the perinatal death group. Moreover, these are not merely opportunistic infections affecting pregnancies already in difficulty for other reasons, because they are more common in group 2, in which no clear-cut morphologic causes of death were found.

When groups 1 and 2 are compared, two facts emerge: First, infection appears to be more significant in group 2 where no other cause of intrauterine death is found. Second, although to a lesser degree, infection also occurred in group 1, in which 44.4% had evidence of inflammation and organisms were found in 40.0%. Interestingly, 20% of group 1 infections were associated with bacterial etiologies, with 50% having inflammation, and not with myoplasma, in which 0% had inflammation and only 14.3% had positive serology (55.8% in group 2). Thus the low incidence of mycoplasma detected in group 1 culture-negative cases may be due to positive transfer of antibodies from the mother.

The types of microorganisms detected in the perinatal death group consisted of the more pathogenic strains of bacteria in 27.3% of cases ($p = 0.0322$), genital mycoplasmas, primarily *U. urealyticum*, in 78.8% of cases ($p < 0.001$), and viruses in 20.0%. There was no strong evidence that chlamydia caused infection in pregnancy, leading to pregnancy loss in this study. Although there were only 12 bacteria-positive cases, our observations support the concept that certain bacterial infections are associated with pregnancy loss. When cultures for fastidious anaerobes are included, this incidence might be higher; however, in only 1 of 33 cases was inflammation found, with no organisms detected. In a second case bacteria were detected by histology but were not isolated.

The most significant organism detected was genital mycoplasmas, which were found in 78.8% of perinatal death cases compared to 32.3% of control cases ($p = 0.0142$), particularly among group 2 in which 91.3% of cases had evidence of mycoplasma ($p = 0.004$). The odds ratio was 24 times higher that a mycoplasma-positive case would be in group 2. The detection of elevated antibody reponses to *U. urealyticum* in 45.8% of the fetuses of the perinatal death group compared to 0% of controls showed the most significant correlation ($p = 0.000052$). There was an odds ratio of 52 that fetuses with elevated titers would be in the perinatal death group. Antibody titers were also found significantly elevated among their mothers; 58.8% in group 2 compared to 14.3% in group 1 (with $p = 0.0122$). These facts fit with our previous observations in which we found elevated ureaplasm antibody titers in neonates and their treated mothers with a history of pregnancy loss[10] and in neonates requiring intensive care and their mothers.[11]

Finally, a significant correlation was also observed between inflammation and mycoplasma-positive cases. This fact is important, since only mycoplasmas were found in 32.3% of all perinatal death cases with no other organisms detected and 50% of mycoplasma-positive cases had only mycoplasmas. In the mycoplasma-only cases, inflammation was observed in 72.9% of cases, which was comparable to 75% found in bacteria-positive cases. Fever occurred in 45.5% of the mycoplasma cases, which was slightly higher than the 33.3% found in bacteria-positive cases. A correlation between the presence of mycoplasmas and inflammation in the placenta[5, 8] and in fetal lungs[3, 5, 8, 11] has been reported previously. Our evidence of inflammation and elevated antibody responses in the fetus add strength to the concept that genital mycoplasmas cause infection in utero leading to death.

The causality of genital mycoplasmas has been difficult to prove in humans, mainly because of high colonization rates in normal patients. However, in the bovine model, *Ureaplasma diversum* was found to be associated with stillbirth, prematurity, and lung disease of the calf. One specific serotype, D48, is found most often. When the isolate from a field abortion was inoculated into ureaplasma-free pregnant heifers, *U. diversum* was shown to cause stillbirths and prematurity associated with neonatal respiratory disease.[16] These experiments also support the concept that ureaplasmas are pathogenic in utero. In humans, our observations indicate that only certain serotypes of *U. urealyticum* elicit antibody responses in pregnancy loss[10] and neonatal disease.[11] Thus certain serotypes among ureaplasmas may be more pathogenic than others. In addition, many patients may be colonized but not infected.

Since 1970, evidence has suggested that genital mycoplasmas are associated with infections in pregnancy leading to poor outcomes,[5] but because there is a high colonization rate in normal women and neonates,[5] these observations are difficult to interpret. More recent studies have shown that in women with *U. urealyticum*– or *M. hominis*–positive placentas there is a significantly higher incidence of chorioamnionitis, stillbirth, prematurity, neonates requiring intensive care, and neonatal death[6, 7] than among women with mycoplasma-negative placentas. In a recent study genital mycoplasmas were isolated from the placenta in 21% of 144 cases of stillbirth and infant death of up to 54 days of age.[7] We observed an incidence of 39.4% mycoplasma culture–positive cases, which may be higher because we cultured a cube of placenta rather than the chorionic surface and cultured the fetal lung. We also included neonates up to 2 days of age. When we included evidence of *U. urealyticum* based on antibody titers of $\leq 1:64$ to at least one serotype, the incidence of mycoplasmas in cases of perinatal death rose to 78.8%.

In our previous studies of mycoplasma-associated loss we found evidence of inflammation in the placenta and fetal lung and loss of ciliation in the tracheal epithelium,[8] significant elevation of specific antibody titers to eight serotypes of *U. urealyticum* in fetus/neonates and their mothers with poor reproductive histories compared to the control populations,[10, 11] and a reduction in the rate of pregnancy loss in mycoplasma-positive women treated with appropriate antibiotics prior to or during gestation.[9] Among neonates with respiratory failure a higher mortality rate occurred among antibody positive cases.[11] *U. urealyticum* antigen was detected by immunofluorescence in the lung of a term neonate born with *Ureaplasma* pneumonia, and the presence of a high titer of IgM-specific antibody to *U. urealyticum* was observed at 24 hours of age (unpublished observations). These observations strongly support the concept that subclinical mycoplasma infection may be associated with pregnancy loss.

If infection plays a major role in stillbirth, why do pathologists not find any evidence of inflammation? The placenta is only rarely sent for histology when the fetus is autopsied and almost never in cases of perinatal death. It is the placenta that provides the strongest evidence of inflammation and is most suitable for culture. Adequate histologic examination of tissues is required. We found that inflammation was patchy and occurred in only one or two lobes of the lung (unpublished observations), and therefore needs careful examination of several sections. In many cases of intrauterine death, the tissues look autolyzed and sections are seldom taken. In this study in only one case was the placenta and lung tissue so autolyzed as to make recognition of any histologic features impossible. The placenta was autolyzed in 1 of 31 cases and the fetal lung in 5 of 30 cases. If the appearance of the tissues appear to be completely normal, few if any sections may be taken, especially of the placenta.

The supporting microbiologic evidence is often not acquired because adequate specimens are not submitted for culture and serology. Proper cultures of placenta and lung tissue are rarely made for bacteria, mycoplasmas, viruses, and chlamydia. Instead of a surface swab, a large piece of placenta should be surface-sterilized and sampled from inside and a piece of lung tissue removed with sterile precautions. Facilities to perform the mycoplasma and virus antibody studies on sera from mother and fetus/neonate or cord are not readily available. From this study good histology and proper cultures for bacteria, mycoplasma, and viruses would provide adequate support of infection in most cases. Microbiologic results are important in understanding the cause of infection and in the future management of the patient.

A number of explanations are possible on the role of mycoplasma infection in pregnancy. First, genital mycoplasmas may truly be a cause of infection in pregnancy, leading to abortion, stillbirth, and neonatal death. This concept is supported by this study and numerous other studies.[3-5] Second, mycoplasmas may be affecting the maternal or fetal host in such a way that they cannot cope with other bacterial, viral, or chlamydial infections, including currently nonsignificant bacterial species. Third, mycoplasmas may be causing premature delivery which, in association with other morphologic defects or infection, leads to the death of the infant. In our previous studies of spontaneous pregnancy loss, women treated for mycoplasma infection delivered 27 of 32 neonates at term despite extensive histories of premature delivery.[9]

Finally, the presence of mixed microorganisms proved to be very important in understanding the role of infection in perinatal death. In greater than 50% of cases more than one organism was detected. Only by comprehensive microbial studies will we be able to establish which bacteria, alone or in conjunction with other organisms, are truly significant as pathogens in pregnancy and fetal death.

This study strongly supports the hypothesis that intrauterine infection may be the sole cause of stillbirth and neonatal death in more cases than previously recognized when all microorganisms including mycoplasma are considered. Bacteria and viruses are significant in certain cases, but the major pathogen may be genital mycoplasmas such as *U. urealyticum*. In cases where the lethality of a morphologic abnormality is questionable, infection may be the actual cause or a contributory factor. If these findings of intrauterine mycoplasma infection are borne out, stillbirths and early neonatal deaths in mycoplasma-positive women identified as at risk may be preventable with proper antibiotic therapy.

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Does dihydrotestosterone induce atresia in the hypophysectomized immature female rat treated with pregnant mare's serum gonadotropin?

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This study was originally designed to test the hypothesis that the binding of luteinizing hormone in granulosa cells decreases with atresia. The hypophysectomized immature female rat that was primed with pregnant mare's serum gonadotropin and treated with dihydrotestosterone was used as a model for atresia. Histochemical analysis of acid phosphatase was used as a marker for atresia and topical autoradiography with iodine 125–labeled human chorionic gonadotropin for binding of luteinizing hormone. Histologically, there was no significant difference in atresia, acid phosphatase, or ^{125}I-labeled human chorionic gonadotropin binding in antral follicles between control animals given pregnant mare's serum gonadotropin and animals treated with pregnant mare's serum gonadotropin and dihydrotestosterone. Assessment of the total follicular population, however, showed that dihydrotestosterone at dosages of 1 and 5 mg/kg resulted in decreases in atresia of 48% and 58%, respectively. Although these data disprove our hypothesis, they strongly suggest that dihydrotestosterone decreases follicular atresia by increasing the number of small preantral follicles. (AM J OBSTET GYNECOL 1985;151:250-5.)

Key words: Ovary, atresia, follicle, androgens

Atresia, which is the degeneration of follicles, has been suggested to be due to the influence of androgens.[1] As the follicle degenerates, a number of biochemical changes such as increased acid phosphatase, a lysosomal enzyme, have been observed prior to any histologic evidence of degeneration.[2,3] The developing follicle can degenerate at any stage of growth but the preovulatory follicle can normally continue to ovulation. Since the granulosa cells of such follicles acquire luteinizing hormone (LH) receptors[4] we hypothesized that the binding of LH by granulosa cells would decrease with follicular atresia. A loss of LH receptors could limit the size of the follicular pool proceeding to ovulation and provide an additional marker for the process of atresia. We used the pregnant mare's serum gonadotropin–primed, dihydrotestosterone-treated, hypophysectomized immature female rat model of Bagnell et al.[5] to test this hypothesis.

Material and methods

Immature female Sprague-Dawley rats hypophysectomized at 24 days of age were obtained from Charles River Ltd. (Montreal, Quebec). Animals were maintained on a 12-hour-light/12-hour-dark cycle with Purina rat chow and 10% dextrose plus 0.9% saline available as desired.

Upon arrival at age 30 days, animals were weighed and randomly assigned to one of the three treatment regimens as indicated in Fig. 1: (1) saline and sesame oil, (2) pregnant mare's serum gonadotropin and sesame oil, and (3) pregnant mare's serum gonadotropin and dihydrotestosterone. Three experiments were done. Animals were treated with 1 mg of dihydrotestosterone per kilogram of body weight in experiments 1 and 2, with tissues used for frozen sections and routine histologic examination, respectively (treatment 1, n = 6 and n = 4; treatment 2, n = 5 and n = 5; treatment 3, n = 5 and n = 5), and with 5 mg of dehydrotestosterone per kilogram of body weight in experiment 3 (treatment 2, n = 5; treatment 3, n = 9). The first injection of pregnant mare's serum gonadotropin or vehicle was given at 10:00 AM on day 30 and the second injection of dihydrotestosterone or vehicle, 30 hours later at 4:00 PM on day 31. All animals were weighed 24 hours later, decapitated, and blood was collected. Sera were stored for progesterone analysis.

Heads were retained for verification of the completeness of hypophysectomy. Ovaries and uteri were weighed separately. Ovaries were then quick-frozen and stored in liquid nitrogen or fixed in Davidson's fixative for routine histologic analysis.

Alternate frozen sections, 8 μm, were treated for enzyme histochemistry or topical autoradiography. The

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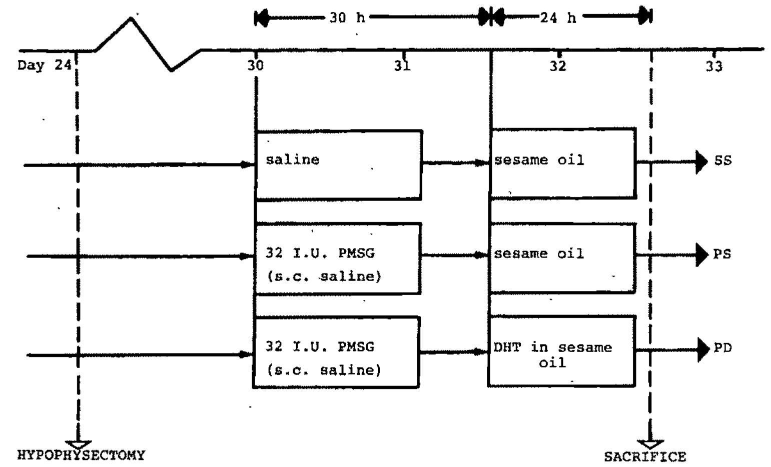

Fig. 1. Experimental design. *PMSG* = Pregnant mare's serum gonadotropin; *DHT* = dihydrotestosterone; *SS* = animals treated with saline and sesame oil (group 1); *PS* = animals treated with *PMSG* and sesame oil (group 2); *PD* = animals treated with *PMSG* and *DHT* (1 and 5 mg/kg) (group 3).

Table I. Mean percent of positive reaction by antral follicles for acid phosphatase grades 1 and 2, and ^{125}I-labeled hCG uptake grades 1 and 2, in frozen tissue sections

Treatment group	*Acid phosphatase in granulosa cells*			*Granulosa cell silver grain localization*			*Follicles with both acid phosphatase and silver grain localization*
	1	*2*	Σ *(1 + 2)*	*1*	*2*	Σ *(1 + 2)*	
1 (n = 6)	0.15	21.38	21.53 ± 15.90	0.28	15.58	15.90 ± 9.17	9.47 ± 8.08
2 (n = 6)	27.37	53.57	80.92 ± 4.04	22.60	33.10	55.62 ± 9.57	50.33 ± 9.64
3 (n = 5)	30.46	49.42	79.92 ± 13.14	22.74	34.24	56.84 ± 6.97	52.66 ± 10.26

Kruskal-Wallis analysis of variance showed a significant ($p < 0.01$) interacton between the three treatment groups. Mann-Whitney U test ($p < 0.05$ to $p < 0.001$) indicated that the differences were significant between groups 2 and 3 and group 1. No significant differences were found between groups 2 and 3.

presence of acid phosphatase was demonstrated by the method of Pearse[6] but with an acetate buffer, pH 5.0. Control sections were incubated without substrate. Sections for autoradiography were incubated for 60 minutes at 37° C with approximately 20,000 cpm of ^{125}I-labeled human chorionic gonadotropin (hCG) solution (New England Nuclear, Lachine, Quebec; specific activity, 58.1 μCi/μg). hCG was used instead of rat LH because it is easier to obtain in pure form and has the same biologic activity as LH. Control autoradiographic sections were incubated with a 3000-fold excess of cold hCG (A.P.L. 10,000 USP, Ayerst Laboratories, Montreal, Quebec). After incubation, sections were fixed in Bouin's fixative and dipped in Kodak NTB-2 Nuclear Track Emulsion and allowed to expose for 5 weeks at −20° C, at which time they were photographically developed.

Radioimmunoassay. Radioimmunoassay of serum progesterone was performed with a progesterone antiserum prepared by the method of Armstrong et al.[7] The antiserum had a cross-reactivity of <0.1% for dihydroprogesterone, testosterone, dihydrotestosterone, androstenedione, estradiol, and estrone and 1.2% for corticosterone.

Quantitation. All slides of frozen sections were randomly assigned a code number and five sections were selected for quantitation from each animal in all three treatment groups. Histochemical sections were consulted for follicular reactivity to the lysosomal enzyme acid phosphatase. A grade 2–positive reaction was assigned if a homogeneous distribution of a bright red color was present in the granulosa cell layer of the follicle. A negative reaction (0) was considered to be a dull brown-yellow color equivalent to the color of the stromal tissue on the control incubation slide. Follicles showing some red coloration but of variable distribution within the granulosa cell layer and of lesser degree were considered to be positive and given a score of 1.

Matching autoradiographic sections were similarly analyzed for the localization and degree of silver grain deposition within the granulosa cell layer: follicles showing definite dense black graining were graded as 2; those showing levels not above background were considered to be 0; and those showing intermediate

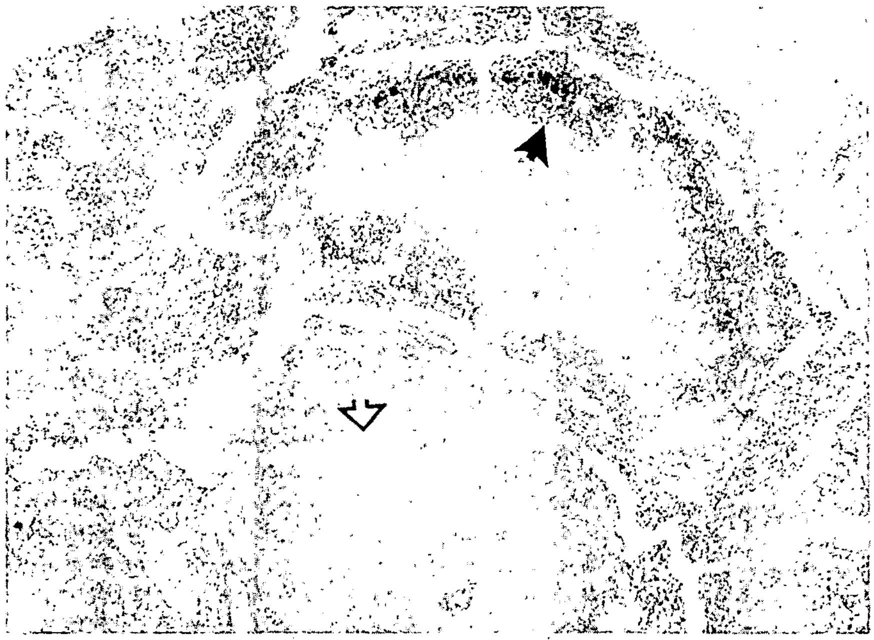

Fig. 2. Photomicrograph illustrates a typical positive reaction for acid phosphatase *(black arrow)*. Control sections were much lighter in color and comparable to areas indicated by *white arrow*. (× 100.)

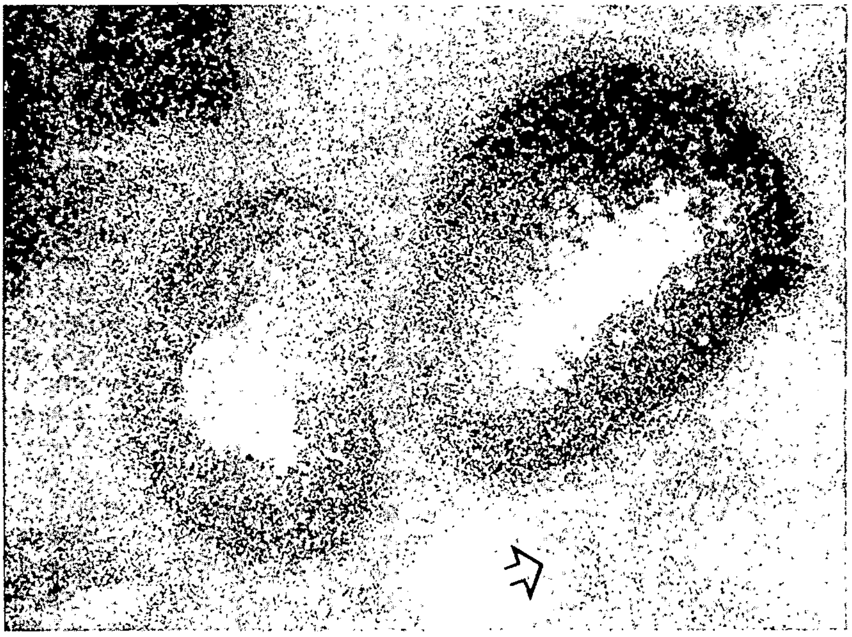

Fig. 3. Photomicrograph illustrates typical autoradiograph of sections incubated with ^{125}I-labeled hCG. Background activity was similar to area indicated by *white arrow*. (× 100.)

levels were rated as 1. A follicle not present in slides for either manipulation was not included in the count.

To establish follicle numbers, development, and health, every fifth routine histologic section of each ovary was analyzed by counting the number of follicles exhibiting an oocyte nucleus in cross section. Development was classified by Pedersen and Peters'[8] scheme based on the mouse and the health of the follicle was assessed by Byskov's[9] morphologic criteria for atresia, where appropriate. Of the developmental classification, the primordial follicles constituted classes 1 to 3B with up to 60 granulosa cells; the preantral follicles, classes 4 to 5B with up to 400 granulosa cells; and the antral group, classes 6 to 8 with granulosa cells >400. Classifications 5A to 8 were considered to be equivalent to the antral follicles evaluated in frozen sections. These

Table II. Percentage of atresia in ovaries of animals treated with pregnant mare's serum gonadotropin and dihydrotestosterone

Class	*Experiment*	*Treatment group 1*	*Treatment group 2*	*Treatment group 3*
Follicular classes 5A-8	1 and 2	89.23 ± 1.69 (n = 4)	89.48 ± 2.71 (n = 5)	80.58 ± 2.92 (n = 5)
	3	—	90.94 ± 3.31 (n = 5)	88.90 ± 2.70 (n = 9)
Overall atresia classes 1-8	1 and 2	49.00 ± 5.90 (n = 4)	53.40 ± 5.82 (n = 5)	27.80 ± 4.96 (n = 5)
	3	—	27.20 ± 4.55 (n = 5)	11.40 ± 0.65 (n = 9)

Kruskal-Wallis analysis of variance indicated that there was a significant interaction ($p < 0.05$) in overall atresia between treatment groups in the combined data of experiments 1 and 2. Mann-Whitney U test showed that the difference ($p < 0.001$) was significant between treatment groups 2 and 3.

Table III. Number of follicles per classification in each ovary of animals treated with pregnant mare's serum gonadotropin and dihydrotestosterone

Treatment group	*Experiment*	*Follicular class*										*Total*
		1	*2*	*3A*	*3B*	*4*	*5A*	*5B*	*6*	*7*	*8*	
1	2 (n = 4)	None	34.75 ±11.90	68.50 ±4.63	8.25 ±2.25	5.75 ±0.85	24.25 ±6.20	1.25 ±0.75	0.25 ±0.25	None	None	143.00 ±14.21
2	2 (n = 5)	None	25.80 ±7.14	68.20 ±15.44	9.60 ±2.69	7.00 ±1.87	33.60 ±7.27	2.40 ±0.81	10.40 ±3.28	6.60 ±2.01	2.00 ±0.55	165.60 ±34.62
	3 (n = 5)	None	51.60 ±34.23	140.20 ±50.00	6.20 ±2.23	3.40 ±1.17	21.60 ±6.30	0.83 ±0.31	3.00 ±1.05	3.00 ±1.14	2.00 ±0.32	232.00 ±89.23
3	2 (n = 5)	0.20 ±0.20	67.00 ±22.67	101.00 ±21.18	8.60 ±2.32	3.60 ±1.21	24.20 ±3.54	2.80 ±1.11	6.40 ±2.11	4.80 ±1.16	1.60 ±1.03	220.20 ±45.05
	3 (n = 9)	None	94.89 ±20.35	299.69 ±29.73	6.67 ±1.58	7.33 ±2.75	27.44 ±4.53	2.22 ±0.62	4.44 ±0.75	4.78 ±0.88	3.56 ±0.93	451.00 ±37.90

Follicular classes 1 to 3B were primordial; 4 to 5B, preantral; and 6 to 8, antral. With the Mann-Whitney U test significant differences in numbers of follicles were found in class 3A between treatment groups 2 and 3 ($p < 0.05$, experiment 3); in class 6, between treatment group 1 and those of treatment groups 2 and 3 ($p < 0.01$, experiment 2); in class 7, between treatment group 1 and those of treatment groups 2 and 3 ($p < 0.01$, experiment 2); and in total follicles, between treatment group 2 and group 3 ($p < 0.001$, experiment 3).

data were then used to determine the percent of follicular atresia.

Statistical analysis. All data were expressed as the mean ± SEM. For all data, $p = 0.05$ was taken as the level of significance.

One-way analysis of variance and paired and unpaired *t* tests were used to analyze body and organ weights and serum progesterone levels. Nonparametric data were analyzed with the Kruskal-Wallis analysis of variance and the Mann-Whitney U test. Contingency tables were used to evaluate acid phosphatase reactivity and ^{125}I-labeled hCG localization from which χ^2 was derived.

Results

Body and organ weights. No significant differences in total body weight before and after treatment were found for any group. Ovarian weights and uterine weights were found to be significantly different in treatment groups 1 and 2 but were not significantly different in treatment groups 2 and 3, reflecting the trophic influence of pregnant mare's serum gonadotropin on the ovaries (data not shown).

Acid phosphatase and topical autoradiography for ^{125}I-labeled hCG. The percent of positive reactivity for antral follicles is shown in Table I. Significant differences were found between groups 1 and 2 and 1 and 3 but not between 2 and 3 for the number of antral follicles with positive acid phosphatase reactivity, granulosa cell silver grain localization, or for those follicles exhibiting both. The number of follicles with both ^{125}I-labeled hCG binding and positive acid phosphatase correlated more closely with hCG binding. Fig. 2 illustrates a section incubated with the substrate sodium α-naphthyl phosphate for the histochemical demonstration of acid phosphatase. Similarly, Fig. 3 shows a section incubated with ^{125}I–labeled hCG solution for the autoradiographic localization of LH binding.

The presence of a positive reaction for acid phosphatase apparently correlated well with localization of

Table IV. Serum progesterone (nanograms per milliliter) in pregnant mare's serum gonadotropin–treated rats

Experiment	*Treatment group 1*	*Treatment group 2*	*Treatment group 3*
1 and 2 (dihydrotestosterone, 1 mg/kg)	0.44 ± 0.57 (n = 10)	1.69 ± 0.94 (n = 11)	2.13 ± 1.07 (n = 10)
3 (dihydrotestosterone, 5 mg/kg)	—	1.62 ± 0.56 (n = 5)	1.06 ± 0.16 (n = 9)

Unpaired *t* test: $p < 0.001$ between treatment groups 2 and 3 and group 1; $p < 0.05$ between experiments 1 and 2 and experiment 3 in treatment group 3. No other difference was significant.

silver grains over the granulosa cell compartment for treatment groups 2 and 3, with χ^2 values of 103.30 and 173.32. Treatment group 1 had a χ^2 of 212.56. Ovaries from this group showed a concentration of silver grains in the medullary region, as opposed to a more diffuse pattern for groups 2 and 3.

Histologic determination of atresia. Table II gives the percent of atresia for the experiments where 1 or 5 mg of dihydrotestosterone was used. No significant difference in the incidence of atresia was seen in follicles classified as 5A to 8 for groups 1, 2, and 3. By contrast, analysis of the overall incidence of atresia within the total follicular population showed a significant decrease in group 3 compared to group 2. Animals treated with 5 mg of dihydrotestosterone also had a significantly lower incidence of atresia (42%) than those treated with 1 mg (52%).

The number of follicles per classification group is shown in Table III. The total number of follicles counted ranged from 143 to 451. A significantly greater number of follicles of class 3A was found in group 3 in experiment 3 where 5 mg of dihydrotestosterone was used. This group also had the largest total number of follicles. Significantly more follicles of types 6 and 7 were found in groups 2 and 3, compared to group 1.

Serum progesterone. Table IV shows the serum progesterone concentrations. Pregnant mare's serum gonadotropin increased serum progesterone levels in all groups. Although it appeared that dihydrotestosterone had differential effects on pregnant mare's serum gonadotropin–increased progesterone levels, these levels were not significantly different from those achieved with pregnant mare's serum gonadotropin alone. However, progesterone levels with 5 mg of dihydrotestosterone were lower than those with 1 mg.

Comment

The binding of LH to ovarian follicles has been shown to decrease in advanced atresia when analyzed for specific binding of radiolabeled LH in the sodium pentobarbital–treated proestrous rat[10] or the estrous ewe.[11] Similar findings were reported in the degenerating preovulatory rat follicle[12] or with induced atresia in hamster follicles[13] by means of topical autoradiographic techniques. Our original objective was to use a histochemical marker for early atresia in frozen sections and to determine in the adjacent section whether ^{125}I-labeled hCG uptake as measured by autoradiographic techniques could be correlated with the histochemical data. As shown in Table I, although there was a good correlation between positive acid phosphatase activity and ^{125}I-labeled hCG uptake in the same antral follicles of 50% of groups 2 and 3, in all three experimental groups the total number of follicles with positive enzyme activity exceeded the total number of follicles with marked density of silver grains. Since acid phosphatase activity has been shown by others[2, 3] to be a good marker for atresia, these data do suggest that there is decreased ^{125}I-labeled hCG binding in granulosa cells of early atretic follicles. hCG is known to bind specifically to the LH receptor of rat ovaries[14]; thus the lack of ^{125}I-labeled hCG binding cannot be explained on the basis of heterologous gonadotropins.

Ideally, it was expected that histologic data, acid phosphatase activity, and ^{125}I-labeled hCG uptake could be correlated on an individual follicle basis. Practically, the frozen sections did not fix and stain satisfactorily to provide sufficient resolution under microscopic examination to allow assessment of atresia. One ovary of each pair in experiments 2 and 3 was therefore fixed and processed separately for histologic examination of atresia by the standard criteria of Byskov.[9] By these histologic criteria, the data in Table II indicate that the overall incidence of atresia was significantly decreased in group 3 animals compared to group 2 animals. This difference is accounted for by the higher percentage of normal follicles of classes 1 to 4 in the dihydrotestosterone-treated animals, since the incidence of atresia in follicles of classes 5A to 8 was similar in groups 2 and 3. The overall atresia rate in experiment 3 was lower than that in experiment 2 and this could be due to differences in shipment of animals—those of experiment 3 were 6 to 11 gm lighter than those of experiment 2 and gained 10 to 13 gm of weight during the treatment period, whereas those in experiments 1 and 2 gained 1 to 4 gm. In spite of this apparent difference in rats, the effects of dihydrotestosterone were dramatically and unpredictably manifested in an overall decrease in atresia in both experiments, strongly suggesting that the model for atresia as described by Bagnell et al.[5] must be viewed with caution. Contamination of the dihydrotestosterone was ruled out by recrystallization of the steroid used and assessment of its melting point, which was identical to that of dihydrotestosterone.

Although we used the identical protocol of Bagnell et al.[5] we were unable to replicate their results in terms of a dihydrotestosterone-influenced increase in the atretic rate. These differences may be due to methodology. We made every attempt to analyze the follicles in an unbiased fashion. Slides were coded and examined by a number that was decoded once all the analyses for one experiment were done. For none of our analyses by histochemistry, autoradiography, or histology were we able to detect an increase in atretic follicles after dihydrotestosterone treatment. Our results correspond somewhat to data reported by Farookhi and Desjardins[15] that in some circumstances dihydrotestosterone does not increase atresia, as evidenced by absence of differences in ovulation rates, but may increase follicular development. This would be compatible with our observations in experiment 3 where there was a threefold increase in the numbers of class 3A follicles after dihydrotestosterone treatment.

In experimentally induced atretic follicles of the hamster there is a shift of steroidogenesis from estrogen to progesterone synthesis.[16] The absence of any significant increase in progesterone levels in the group 3 animals as compared to group 2 animals (Table IV) provides further supporting data that increased atresia is not associated with dihydrotestosterone treatment.

In summary, our data suggest that, contrary to accepted beliefs, dihydrotestosterone may not induce atresia but indeed may stimulate early follicular development.

We are grateful to Drs. A. G. Byskov and A. Hirshfield for assistance in interpreting follicular development.

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Longitudinal measurements of fetal breathing, body movements, heart rate, and heart rate accelerations and decelerations at 24 to 32 weeks of gestation

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Fetal breathing, fetal body movements, fetal heart rate, and fetal heart rate accelerations and decelerations were studied longitudinally in healthy fetuses between 24 and 32 weeks' gestation in the second and third hour following an 800 kcal maternal meal. The expected increase in fetal breathing following a maternal meal was not seen until fetuses were at 30 to 32 weeks' gestation. The number of body movements decreased and the interaction between body movements and fetal heart rate accelerations became more evident as fetuses became older. Fetal heart rate decelerations increased with gestational age, and the relative proportion of total decelerations that were either associated with body movements or were part of a deceleration/acceleration/deceleration complex increased from 24 to 32 weeks' gestation. The data support the hypothesis that gestational age is an important variable to consider when interpreting biophysical measurements in the human fetus at 24 to 32 weeks' gestation. Fetal body movements may be the single most important measurement of fetal health at these gestational ages. (AM J OBSTET GYNECOL 1985;151:256-63.)

Key words: Fetal breathing, fetal body movements, fetal heart rate

The concept of cycles of rest and activity in the human fetus as determined by measurements of fetal heart rate, body movements, and breathing have become well accepted.[1-5] This concept has led to the physiologic basis for the appropriate clinical use of fetal biophysical measurements for the identification of fetal well-being antenatally. These same biophysical measurements have only received preliminary clarification in preterm fetuses.[5-8]

The purpose of the present study was to longitudinally determine normal patterns of fetal breathing, fetal body movements, fetal heart rate, and fetal heart rate accelerations and decelerations in healthy fetuses of normal pregnant women at 24 to 32 weeks' gestation.

Material and methods

Fetal breathing movements, fetal gross body movements, fetal heart rate, and maternal heart rate were recorded simultaneously for 2 hours beginning 1 hour after an 800 kcal breakfast or lunch (Fig. 1) in a group of 11 normal pregnant women studied longitudinally between gestational ages of 24 to 32 weeks. Each woman was studied four consecutive times, once each 24 to 26, 26 to 28, 28 to 30, and 30 to 32 weeks' gestation. The patients were certain of their menstrual dates, and all had had an early pregnancy examination. Their dates were confirmed at the time of each study by a determination of the fetal biparietal diameter.

Fetal breathing movements and gross body movements were recorded with the use of a real-time scanner (ADR, Tempe, Arizona). Individual breaths and gross body movements were identified on a video screen and recorded on a chart recorder with an event marker as previously described.[9] Fetal heart rate was simultaneously recorded by means of a Hewlett Packard 8030A ultrasonic external fetal heart monitor. Maternal heart rate was recorded from three conventional electrocardiogram electrodes placed on the maternal chest.

Each parameter was analyzed independently in consecutive 1-minute epochs. The proportion of each consecutive 1-minute epoch during which fetal breathing movements and fetal gross body movements were present was analyzed to determine the respective incidence of each. Also, the number of fetal gross body movements which occurred during the 2-hour recording period was analyzed. A train of any number of fetal body movements which occurred <6 seconds apart was identified as a single movement. The duration of each movement was also identified. In addition, the intervals

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of time during which no movement was observed was also identified for each 2-hour recording.

The mean fetal heart and maternal heart rates were established and were correlated for each age group. Accelerations and decelerations in fetal heart rate ≥10 bpm from baseline for a minimum duration of 10 seconds were identified. Baseline fetal heart rate was defined as the mean fetal heart rate 3 minutes before and 3 minutes after an observed change. Accelerations and decelerations were then classified into three groups: ≥10 bpm but <15 bpm, ≥15 bpm but <20 bpm, and ≥20 bpm. An epoch was excluded from analysis if it contained >30 seconds (i.e., 50%) of fail time for fetal heart rate analysis or >12 seconds (i.e., 20%) of fail time for fetal breathing and body movement analysis. Accelerations and decelerations were then classified according to whether they were associated with fetal body movements. A heart rate change was classified as being with movement if the movement occurred within 30 seconds of the start of the acceleration or deceleration. Movements were also analyzed to identify those which occurred without accelerations or decelerations. Decelerations were then further classified to include those that were part of an acceleration/deceleration or deceleration/acceleration complex (i.e., deceleration associated with acceleration). Results presented are expressed as the mean plus or minus the standard error, and significance was determined by means of a paired *t* test.

Results

A summary of the patient data and fetal outcome is presented in Table I. It will be noted that the caloric intake at each of the gestational ages studied was not statistically different. All fetuses were normal at birth. Technical and patient interruption fail times for fetal breathing and gross fetal body movements were 2.0%, 4.9%, 0.7%, and 2.5% at 24 to 26, 26 to 28, 28 to 30, and 30 to 32 weeks' gestation, respectively.

Fetal breathing. Fetal breathing in the second and third hour after an 800 kcal maternal meal increased progressively from 5.8 ± 0.7% at 24 to 26 weeks' gestation to 11.3 ± 1.4%, 19.5 ± 1.0%, and 30.5 ± 1.1% ($p < 0.001$) at 26 to 28, 28 to 30, and 30 to 32 weeks' gestation respectively. That is, the fetal breathing response that was normally expected in the second and third hour[1, 9] was not apparent until 30 to 32 weeks' gestation and was totally absent at 24 to 26 and 26 to 28 weeks' gestation (Fig. 2).

Fetal body movements. The mean number of fetal body movements per 2-hour period decreased significantly ($p < 0.05$) from 106.5 ± 9.8 at 24 to 26 weeks' gestation to 76.7 ± 7.9 and 69.3 ± 4.7 at 28 to 30 and 30 to 32 weeks' gestation, respectively (Fig. 3).

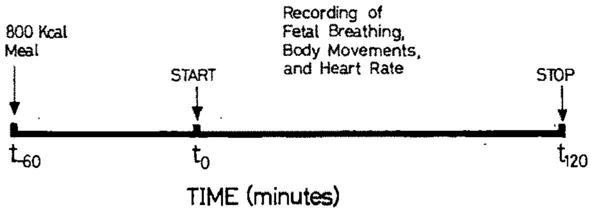
800 Kcal Meal
START
Recording of Fetal Breathing, Body Movements, and Heart Rate
STOP
t_{-60}
t_0
t_{120}
TIME (minutes)

Fig. 1. Design of experiment.

Table I. Summary of patient data and fetal outcomes

Characteristic	*Measurement*
Maternal age (yr)	26.2 ± 0.6
Maternal caloric intake (kcal)	
24-26 weeks	679.1 ± 40.6
26-28 weeks	714.1 ± 71.1
28-30 weeks	764.5 ± 26.6
30-32 weeks	722.8 ± 26.9
Gestational age at time of study (wk)	
By menstrual age	
24-26 weeks	24.3 ± 0.2
26-28 weeks	26.9 ± 0.2
28-30 weeks	28.9 ± 0.2
30-32 weeks	30.8 ± 0.1
By biparietal diameter	
24-26 weeks	24.3 ± 0.4
26-28 weeks	27.3 ± 0.2
28-30 weeks	29.2 ± 0.2
30-32 weeks	31.5 ± 0.2
Gestational age at delivery (wk)	40.0 ± 0.3
Infant birth weight (gm)	3734.1 ± 145.0
5-Minute Apgar score	9.0 ± 0.4

The percent time spent making fetal body movements in the second and third hour following an 800 kcal maternal meal was 10.7 ± 0.8, 9.5 ± 0.5, 7.0 ± 0.5, and 6.8 ± 0.5 at 24 to 26, 26 to 28, 28 to 30, and 30 to 32 weeks' gestation. While the percent time spent making fetal body movements was statistically significantly reduced at 28 to 32 weeks' gestation as compared to 24 to 28 weeks' gestation ($p < 0.01$), the differences were not large.

The frequency distribution of the duration of movements was not statistically different from 24 to 32 weeks' gestation (Fig. 4). Approximately 30% of all movements were >5 seconds but ≤15 seconds in duration in all gestational age groups, and only 4.6%, 4.3%, 3.1%, and 5.9% of all movements were >15 seconds' duration at 24 to 26, 26 to 28, 28 to 30, and 30 to 32 weeks' gestation, respectively. Examination of consecutive epochs of time in which no movement occurred demonstrated that the longest interval of time without a movement occurring in 95% of the intervals studied was 6 minutes at 24 to 28 weeks' gestation and 10 minutes at 28 to 32 weeks' gestation (Fig. 5).

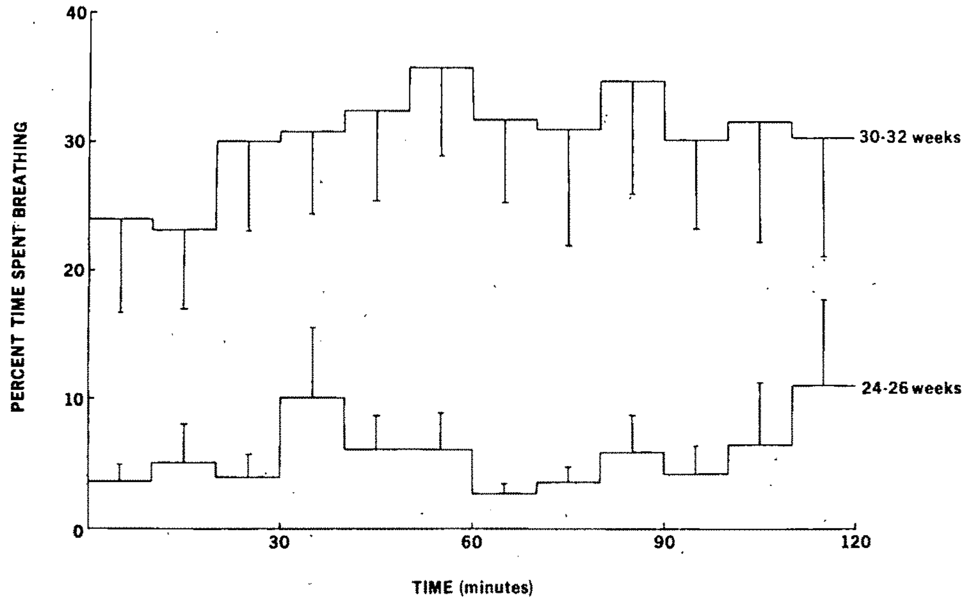

Fig. 2. The incidence of fetal breathing activity plotted in 10-minute epochs per 2 hours at 24 to 26 and 30 to 32 weeks' gestation. The percentage of time spent making fetal breathing movements increased significantly between these gestational ages ($p < 0.001$).

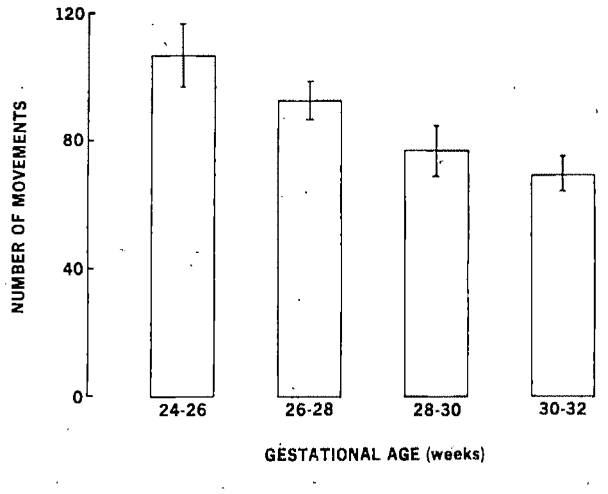

Fig. 3. The mean number of fetal body movements at 24 to 32 weeks' gestation ($p < 0.05$).

Fetal heart rate. The mean 2-hourly fetal heart rate decreased from 148.8 ± 0.3 bpm at 24 to 26 weeks' gestation to 144.3 ± 0.5 bpm at 30 to 32 weeks' gestation ($p < 0.01$). The mean heart rate during epochs of movement (Fig. 6), while decreasing with gestational age, was consistently higher as compared to epochs of time with no movement ($p < 0.01$).

Examination of the correlation coefficient between the mean 2-hourly maternal and the mean 2-hourly fetal heart rate was 0.5, 0.62, 0.52, and 0.11 at 24 to 26, 26 to 28, 28 to 30, and 30 to 32 weeks' gestation. This relationship was only statistically significant for fetuses at 26 to 28 weeks' gestation (Figs. 7 and 8).

Fetal heart rate accelerations and decelerations. The number of fetal heart rate accelerations increased significantly from 7.6 ± 1.2 at 24 to 26 weeks' gestation to 21.9 ± 3.9, 21.3 ± 2.3, 21.5 ± 1.9 at 26 to 28, 28 to 30, and 30 to 32 weeks' gestation, respectively ($p < 0.001$) (i.e., after 26 weeks they remained constant). Analysis of the frequency distribution of the amplitude of accelerations (Fig. 9) showed that as fetuses became older, the percent of total accelerations <15 bpm decreased significantly from 72.4% of total accelerations at 24 to 26 weeks' gestation to 52.1% at 30 to 32 weeks' gestation ($p < 0.01$). The proportion of accelerations ≥15 bpm increased significantly from 27.6% of total accelerations at 24 to 26 weeks' gestation to 47.9% at 30 to 32 weeks' gestation ($p < 0.01$). Similarly, accelerations ≥20 bpm increased significantly from 0 at 24 to 26 weeks to 22.8% of total accelerations at 30 to 32 weeks' gestation ($p < 0.01$).

At 24 to 26 weeks' gestation 18.4% of all accelerations were not associated with fetal body movements. This remained relatively constant to 32 weeks' gestation. It was of note that the vast majority of these accelerations were <15 bpm with only 3.2% to 5.7% of them being ≥15 bpm and therefore clinically recognizable. That is, at 30 to 32 weeks' gestation, 4.7% of total accelerations were spontaneous occurrences. The remainder of the accelerations (Fig. 10) occurred with fetal body movements. Of those, 57.9%, 47.5%, 39%, and 40.9% were <15 bpm in amplitude at 24 to 26, 26 to 28, 28 to 30, and 30 to 32 weeks' gestation, respectively. That is, nearly 60% of total accelerations that were associated with body movements at 24 to 26 weeks' gestation and 41% of total accelerations at 30 to 32 weeks' gestation

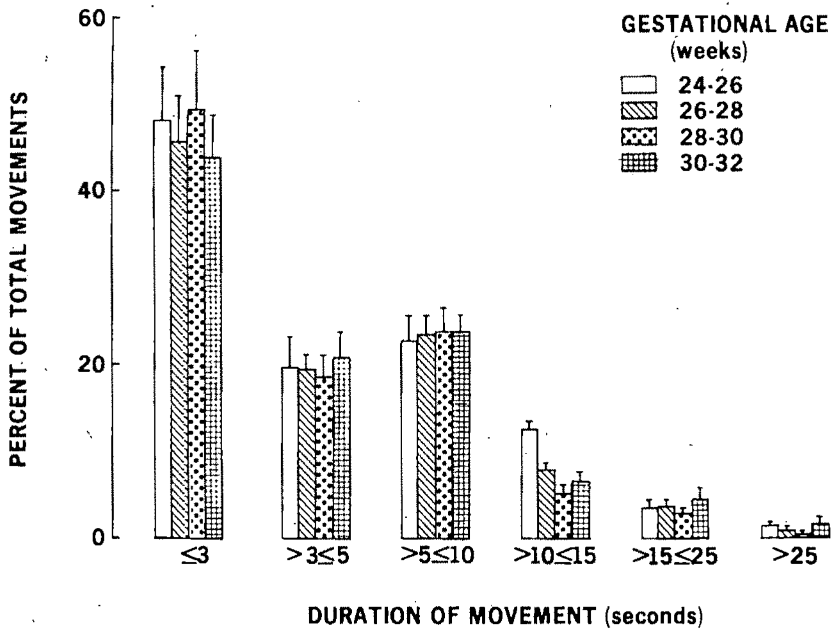

Fig. 4. Frequency distribution of the duration of fetal body movements at 24 to 32 weeks' gestation.

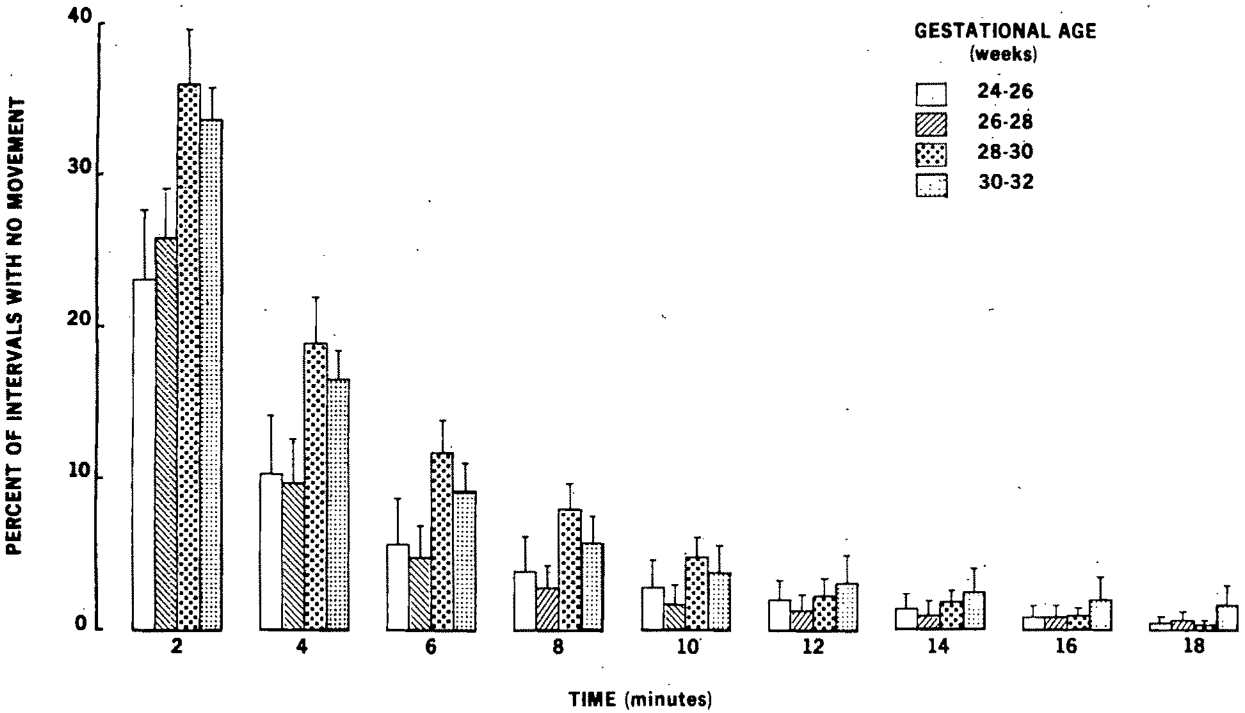

Fig. 5. Percentage of intervals with no fetal body movements per 2 hours at 24 to 32 weeks' gestation.

were not clinically recognizable as fetal heart rate accelerations associated with body movements ($p < 0.05$). Finally, 30% of the total number of movements were not associated with a fetal heart rate acceleration from 24 to 32 weeks' gestation. That is, between 24 to 32 weeks' gestation, 18.4% of total accelerations were not associated with movements and 30% of total movements were not associated with fetal heart rate accelerations.

The mean number of fetal heart rate decelerations increased significantly from 4.2 ± 0.7 at 24 to 26 weeks to 9.3 ± 2.1, 9.4 ± 2.0, and 8.5 ± 2.1 at 26 to 28, 28 to 30, and 30 to 32 weeks' gestation, respectively ($p < 0.05$). Examination of the frequency distribution of the amplitude of decelerations (Fig. 11) showed that decelerations <15 bpm accounted for approximately 60% of all decelerations and that ≥15 bpm accounted for approximately 40% of all decelerations in all ges-

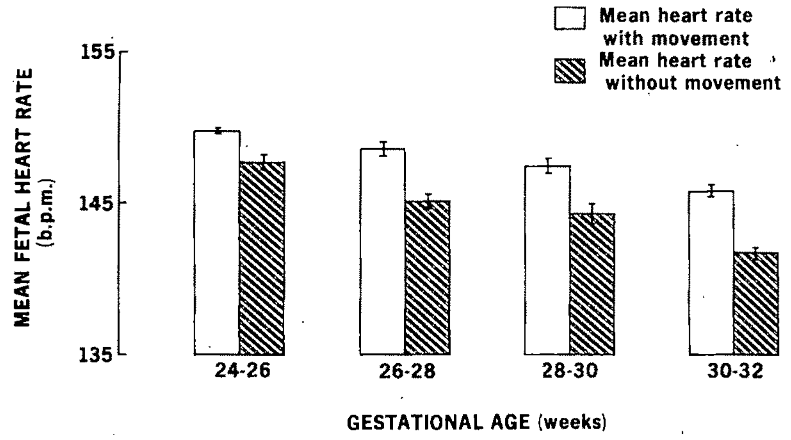

Fig. 6. Mean fetal heart rate during epochs of fetal body movements compared to epochs with no fetal body movements at 24 to 32 weeks' gestation ($p < 0.001$).

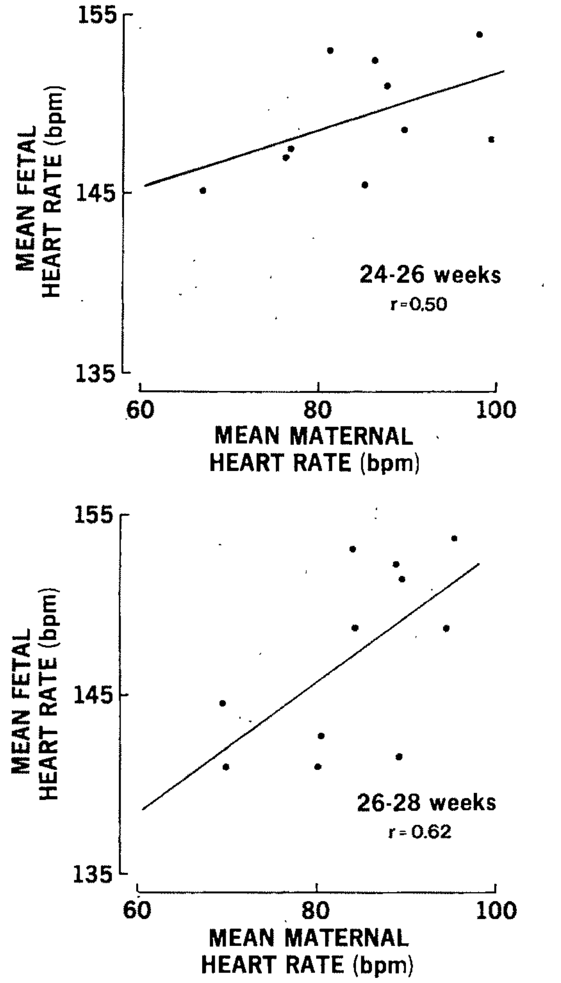

Fig. 7. Correlation of fetal and maternal heart rate per 2 hours at 24 to 26 weeks' *(top)* and 26 to 28 weeks' *(bottom)* gestation.

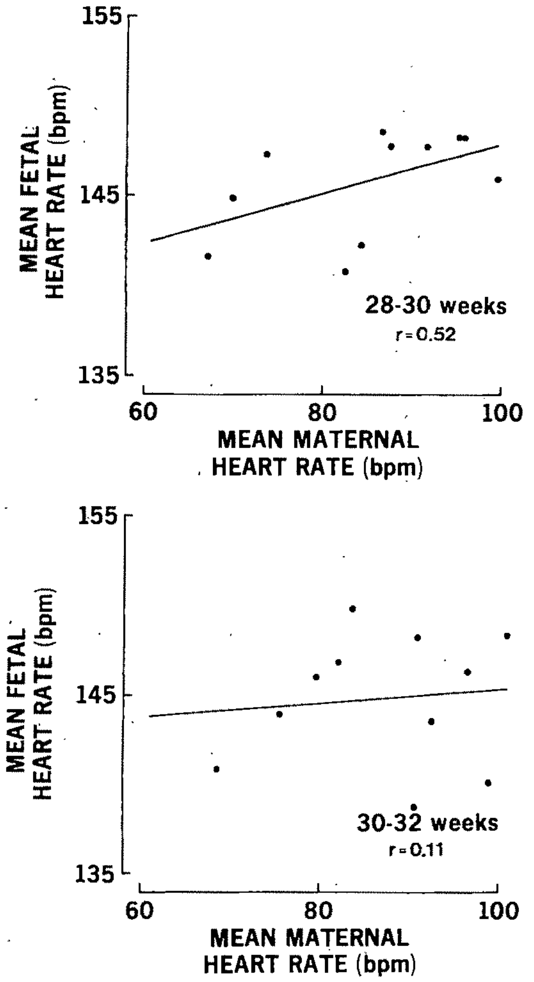

Fig. 8. Correlation of fetal and maternal heart rate per 2 hours at 28 to 30 weeks' *(top)* and 30 to 32 weeks' *(bottom)* gestation.

tational ages between 24 and 32 weeks. It was of note that consistently about 15% of all decelerations were profound and >20 bpm between 24 and 32 weeks' gestation.

The percent of fetal heart rate decelerations associated with movement tended to increase with gestational age (not statistically significant). At 24 to 26 weeks' gestation, 35.7% of all decelerations were associated with

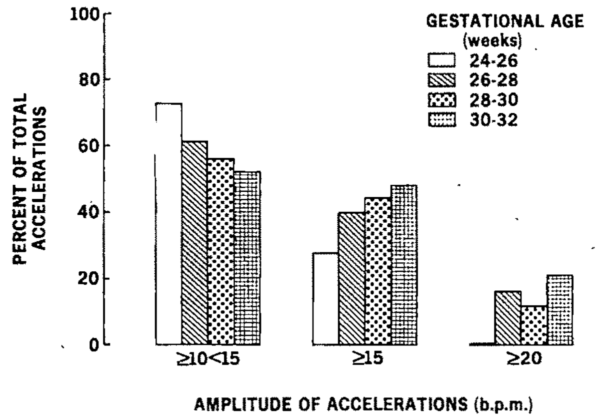

Fig. 9. Frequency distribution of the amplitude of fetal heart rate accelerations at 24 to 32 weeks' gestation. The proportion of fetal heart rate accelerations <15 bpm decreased significantly from 24 to 32 weeks' gestation (72.4%, 61.2%, 55.9%, 52.1%), whereas the proportion of accelerations ≥15 bpm increased significantly (27.6%, 38.8%, 44.1%, 47.9%), as did those ≥20 bpm (0%, 16.0%, 13.6%, 22.8%) at the same gestational ages ($p < 0.01$).

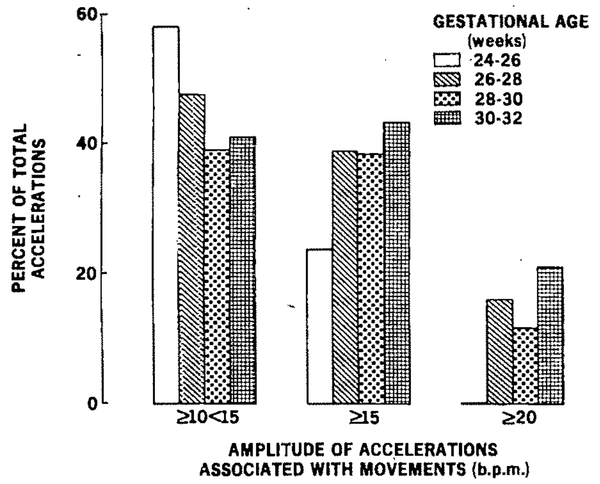

Fig. 10. Frequency distribution of the amplitude of fetal heart rate accelerations associated with fetal body movements. A significantly smaller proportion of fetal heart rate accelerations were <15 bpm and associated with movement between 24 to 32 weeks' gestation (57.9%, 47.5%, 39.0%, 40.9%), whereas accelerations ≥15 bpm and associated with movement increased significantly (23.7%, 35.6%, 38.5%, 43.2%), as did those ≥20 bpm (0%, 16.0%, 11.7%, 20.9%) ($p < 0.05$).

fetal body movements. This association increased to 47.4%, 50.1%, and 51.8% at 26 to 28, 28 to 30, and 30 to 32 weeks' gestation but was not statistically significant (Fig. 12).

The proportion of decelerations associated with an acceleration increased with gestational age ($p < 0.01$). At 24 to 26 weeks' gestation, only 19.1% of all decelerations were associated with an acceleration, whereas 49.5% of all decelerations were associated with an acceleration at 30 to 32 weeks' gestation ($p < 0.001$) (Fig. 13). The number of spontaneous decelerations decreased from 45.2% at 24 to 26 weeks' gestation to 32.8% of total decelerations at 30 to 32 weeks' gestation. That is, spontaneous decelerations in fetal heart rate are more commonly observed at 24 to 26 weeks' gestation as compared to older fetuses.

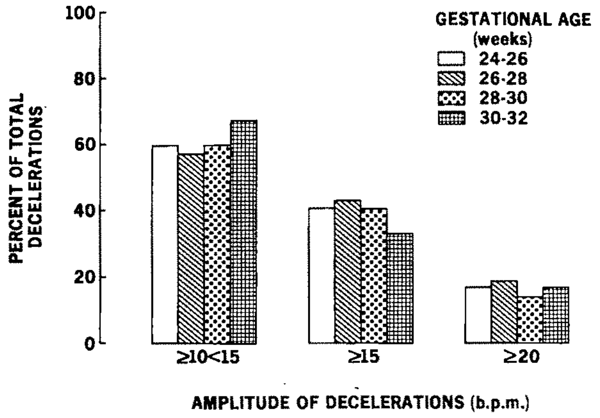

Fig. 11. Frequency distribution of the amplitude of fetal heart rate decelerations at 24 to 32 weeks' gestation. The proportion of decelerations <15 bpm was not significantly different (59.5%, 57.0%, 59.6%, 67.1%) nor were the proportions ≥15 bpm (40.5%, 43.0%, 40.4%, 33.0%) or those ≥20 bpm (16.7%, 18.3%, 13.8%, 16.5%) across gestational ages.

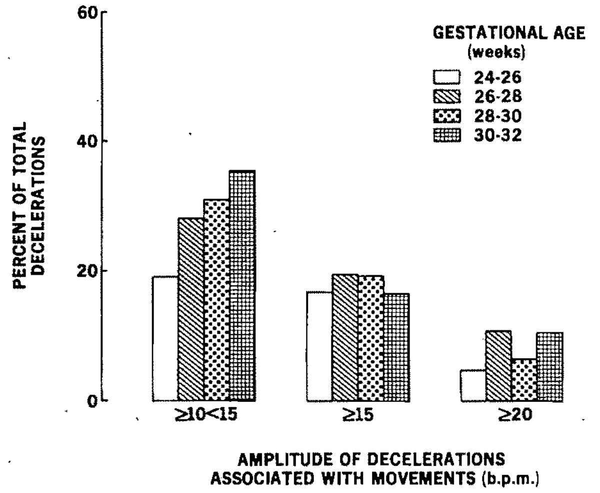

Fig. 12. Frequency distribution of the amplitude of fetal heart rate decelerations associated with fetal body movements at 24 to 32 weeks' gestation. The proportion of decelerations with movement <15 bpm was not statistically different (19.0%, 28.0%, 30.9%, 35.3%), nor were the proportions ≥15 bpm (16.7%, 19.4%, 19.2%, 16.5%) or those ≥20 bpm (4.8%, 18.3%, 6.4%, 10.6%) across gestational ages.

Comment

Long-term simultaneous recording of fetal body movements, fetal breathing, and fetal heart rate in the last 10 weeks of pregnancy has established the existence

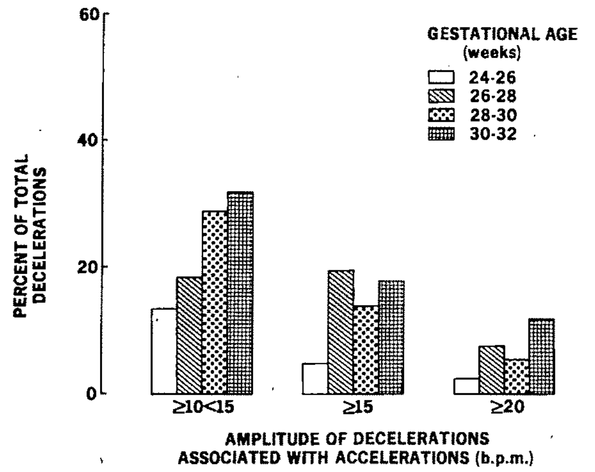

Fig. 13. Frequency distribution of the amplitude of fetal heart rate decelerations associated with a fetal heart rate acceleration at 24 to 32 weeks' gestation. The proportion of decelerations <15 bpm associated with an acceleration increased significantly (14.3%, 18.3%, 28.7%, 31.8%), as did those ≥15 bpm (4.8%, 19.3%, 13.8%, 17.7%) and those ≥20 bpm (2.4%, 7.5%, 5.3%, 11.8%) across gestational ages ($p < 0.05$).

of cycles of rest and activity in the human fetus.[1-5] These periodicities recur about every 80 to 120 minutes.[1-4] Similar periodicities, although alluded to,[5-8] have not been clearly measured in younger fetuses.

In the present studies we found that younger fetuses did not have the expected increase in fetal breathing[1] following a maternal meal until 30 to 32 weeks' gestation, clearly suggesting that periodicities in fetal breathing are not established before 30 weeks' of gestation. Also, the number of movements decreased as fetuses became older; moreover, the longest interval of no movement for 95% of the observation epochs was doubled only by 32 weeks' gestation. This suggests that periodicities in fetal body movements are probably not clearly established in younger fetuses until 30 to 32 weeks' gestation. This is clinically important because, unlike near-term fetuses, fetuses between 24 to 32 weeks gestation are seldom quiet and therefore the prolonged absence of fetal movement may be the best indicator of a sick fetus at these gestational ages.

The mean 2-hourly fetal heart rate decreased significantly with gestational age; however, the mean fetal heart rate during episodes of movements was higher than during episodes of no movement. This suggests that although gestational age has an important effect on mean fetal heart rate,[11, 12] fetal body movements are also important modulators of mean fetal heart rate. This is in agreement with other investigators[5, 12] who have suggested that, during the last half of pregnancy, the fetal heart rate pattern becomes dominated by episodes of low or high heart rate variation and that fetal activity occurs usually in periods of increased heart period variation.[4]

Also, unlike Patrick et al.[12] who found a high correlation between daily mean maternal and fetal heart rate at term, we found a correlation only at 28 to 30 weeks' gestation. The explanation for the difference lies most likely in our short sampling period.

Although we, like others,[5-11] found that the total number of fetal heart rate accelerations increased as gestational age increased, the proportion of larger amplitudes of fetal heart rate accelerations (i.e., ≥15 bpm) increased with gestational age, and the proportion of total heart rate accelerations associated with fetal body movements shifted from low amplitude types of accelerations (i.e., <15 bpm) to accelerations of higher amplitude (i.e., ≥15 bpm) as fetuses reached 32 weeks' gestation. Clinically, this is important because low-amplitude accelerations are not recognizable on biophysical tests of fetal well-being.[15-17] Although large-amplitude accelerations with movement may be present in younger fetuses, they made up only 43.2% of total accelerations at 30 to 32 weeks' gestation.

It was also of note that from 24 to 32 weeks' gestation, approximately 20% of all accelerations were not associated with movements and 30% of all movements were not associated with fetal heart rate accelerations. This is different from near-term and term healthy fetuses in which fetal body movements are invariably associated with accelerations in fetal heart rate.[3-5, 12, 14]

Unlike other investigators,[6, 7, 11] we found that the number of decelerations increased with gestational age. We believe this to be the result of different criteria for selecting fetal heart rate decelerations. Others[6, 7, 11] have defined a deceleration as any change below a fetal heart rate baseline of ≥10 bpm without any regard for the duration of the change. We applied the same criteria to define fetal heart rate decelerations that were used to define fetal heart rate accelerations. Using these strict criteria and defining decelerations that occurred as an acceleration complex or with movement, we were unable to demonstrate many spontaneous decelerations beyond 30 weeks' gestation whereas they were commonly seen in the extremely premature fetus.

In summary, the data support the hypothesis that biophysical measurements in the human fetus at gestational ages of 24 to 32 weeks are very different from those in near-term fetuses and suggest that there is a maturational component that must be taken into account when interpreting measurements of fetal breathing, gross body movements, fetal heart rate, and fetal heart rate accelerations and decelerations at these gestational ages. Furthermore, both spontaneous decelerations and accelerations may be present in fetuses at 24 to 32 weeks' gestation although it is unusual to see spontaneous decelerations beyond 30 weeks' gestation. Also, these studies point out that simultaneous measurements of fetal heart rate, fetal body movements, and fetal breathing at 24 to 32 weeks' gestation are required to help elucidate at what gestational age the cycles of rest and activity become clearly demarcated and what the periodicities of these cycles of rest and activity are at these gestational ages. Such data would provide the physiologic basis for meaningful criteria for the use of such measurements in determining fetal health. Because fetal body movements are very sensitive to fetal oxygenation,[18] fetal body movements alone may be the easiest and most reliable biophysical measurement by which to assess fetal health at 24 to 32 weeks' gestation.

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Decreased serum reverse triiodothyronine levels with major weight loss in obese women

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Anorexia nervosa is accompanied by increased serum reverse triiodothyronine levels and decreased serum triiodothyronine levels. We observed 28 women undergoing gastric plication for morbid obesity and found serum reverse triiodothyronine levels to be significantly decreased ($p = 0.018$) from a preoperative mean (±SD) of 0.30 (±0.17) to 0.21 (±0.06) ng/ml after weight loss. As all of our subjects were on semistarvation diets and had a significant weight loss ($p < 0.001$), the observed decrease in reverse triiodothyronine rather than increase (as reported in anorexia nervosa) suggests factors other than weight loss or a semistarvation diet are important in the reverse triiodothyronine increase observed in anorexia nervosa. (AM J OBSTET GYNECOL 1985;151:264-7.)

Key words: Obesity, thyroid hormone, reverse triiodothyronine, weight loss

Anorexia nervosa is characterized by semistarvation and marked weight loss in young women.[1,2] Thyroid hormone metabolism may be reversibly altered in fasting states. Monodeiodination of thyroxine in the liver results in the production of either serum reverse triiodothyronine or serum triiodothyronine. In fasting states such as anorexia nervosa, increased serum reverse triiodothyronine and decreased serum triiodothyronine levels are found after marked weight loss.[1,2]

Another group of women who experience profound weight loss are morbidly obese women who undergo gastric plication and are placed on a semistarvation diet. The aim of the present study was to assess thyroid parameters in these subjects during profound weight loss and semistarvation.

Material and methods

The study group consisted of 28 premenopausal women, with a mean age of 35.6 years, who planned to have gastric plication for control of morbid obesity. These women were a minimum of 100% above their ideal weight with preoperative mean weight ± SD of 128.6 ± 22.4 kg. With the use of a calculation for ideal body weight,[3] the mean weight ± SD above ideal weight (Δ ideal weight) was 71.1 ± 22.3 kg.

All subjects were interviewed and clinical data recorded at the beginning and end of the study. Subjects on regimens of hormonal medications or medications known to influence hormone levels were excluded at the outset of the study. Two subjects received medroxyprogesterone acetate before the study was completed. There were numerous questions dealing with symptoms and signs of thyroid disease asked in the initial and final interview. After operation, all subjects were instructed on at least one occasion concerning their semistarvation diet of 3150 kJ (750 kcal diet). The dietary components was 60% protein, 20% fat, and 20% carbohydrates.

The subjects were weighed and blood samples drawn preoperatively and on three to five occasions over the 2 years of weight loss. The blood was prepared as serum, stored at −20° C, and analyzed in series at the completion of the study. For the purpose of this study the serum sample drawn preoperatively was compared to the sample at the point at which maximum weight loss had occurred in the follow-up period. Serum concentrations of thyroxine, triiodothyronine, reverse triiodothyronine, and thyroid-binding globulin were measured by standard radioimmunoassay techniques.[4,5] For the ratios of reverse triiodothyronine/thyroxine and triiodothyronine/thyroxine all of the data were expressed in units of nanograms per deciliter. For the conversion of thyroxine data, we used the equation:

$$\text{thyroxine, nanomoles per liter, } \times K = \text{thyroxine, nanograms per deciliter}$$

where K = 1.28. The serum reverse triiodothyronine data was converted from nanograms per milliliter to nanograms per deciliter by a factor of 100. Albumin was measured by the bromocresol green dye-dilution technique.[6]

Statistical analysis was carried out with a paired *t* test to compare the significance of changes seen over the period of weight loss.

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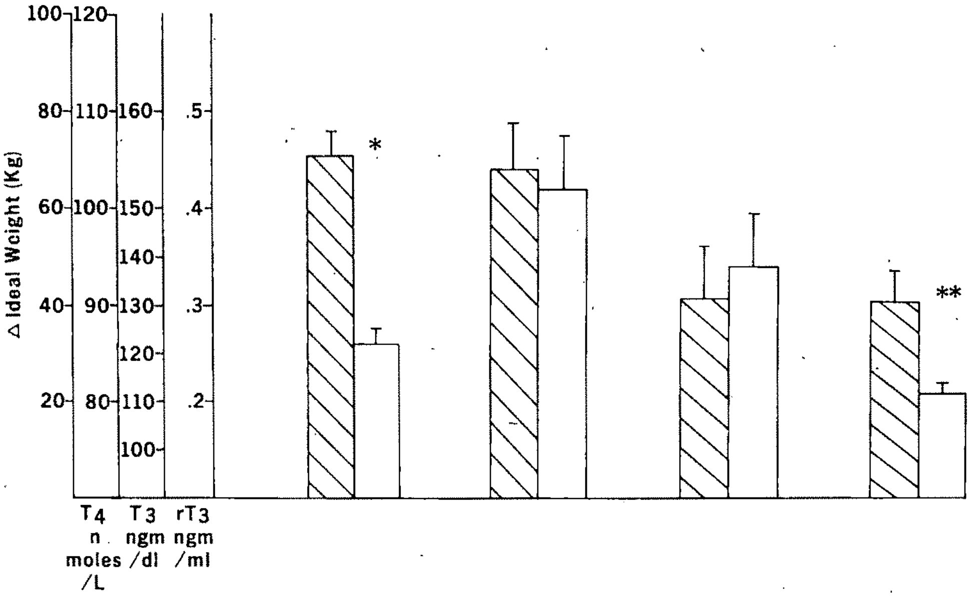

Fig. 1. The mean (± SEM) values of Δ ideal weight (deviation from ideal weight) and serum thyroxine, serum triiodothyronine, and serum reverse triiodothyronine before operation *(hatched)* and after maximum weight loss are expressed. Significant differences were found in the weight loss (*$p < 0.001$) and reverse triiodothyronine values (**$p = 0.018$).

Table I. Values before operation and after maximum weight loss

Time of measurement	*Serum thyroxine (ng/dl)*	*Serum triiodothyronine (ng/dl)*	*Serum reverse triiodothyronine (ng/dl)*	*Reverse triiodothyronine × 100 thyroxine (%)*
Before operation	134 ± 30.8	131.1 ± 27.1	30.4 ± 17	22.7*
After maximum weight loss	131 ± 35.3	138.1 ± 27.8	21.0 ± 5.9	16.0*

*$p = 0.04$.

Results

As illustrated in Fig. 1, a significant decrease ($p < 0.001$) was observed for the mean ± SD Δ ideal weight from 71.1 ± 22.3 kg before operation to 31.6 ± 19.0 kg after weight loss. All 28 women demonstrated consistent decreases in Δ ideal weight over the study period.

A comparison of the thyroid parameters before operation and after maximum weight loss is illustrated in Fig. 1. The serum reverse triiodothyronine decreased significantly from a mean ± SD of 0.30 ± 0.17 ng/ml before operation to 0.21 ± 0.059 ng/ml after weight loss ($p = 0.018$). The coefficient of variation was 57% before operation and 28% after weight loss. The serum triiodothyronine mean ± SD was 127.6 ± 29.0) ng/dl before operation and 136.1 ± 27.7 ng/dl after weight loss. The serum thyroxine level remained unchanged and in the euthyroid range both before operation (mean ± SD, 104.7 ± 24.0 nmol/L) and after weight loss (102 ± 26.5 nmol/L). As the serum reverse triiodothyronine is primarily derived from thyroxine metabolism, the proportion of thyroxine metabolized to reverse triiodothyronine is expressed as a percentage of the ratio reverse triiodothyronine/thyroxine. The reverse triiodothyronine/thyroxine ratio decreased significantly ($p = 0.04$) from 22.7% before operation to 16.0% after maximum weight loss (Table I).

Thyroid-binding globulin levels did not change over the period of weight reduction (Fig. 2). Serum albumin, however, increased significantly ($p < 0.001$) from a preoperative mean ± SD of 40.3 ± 6.6 gm/L to 47.2 ± 3.2 gm/L after weight loss (Fig. 2).

Comment

Plasma thyroxine is converted to triiodothyronine and reverse triiodothyronine through monodeiodination in the liver. Chopra[7] reported that 97.5% of serum reverse triiodothyronine results from this peripheral monodeiodination. It is not known what factors determine in what proportion triiodothyronine and reverse triiodothyronine are produced from thyroxine.

In acute illness involving protein malnutrition, such as anorexia nervosa, the serum triiodothyronine level is decreased after weight loss and a reciprocal increase in serum reverse triiodothyronine is seen. As serum

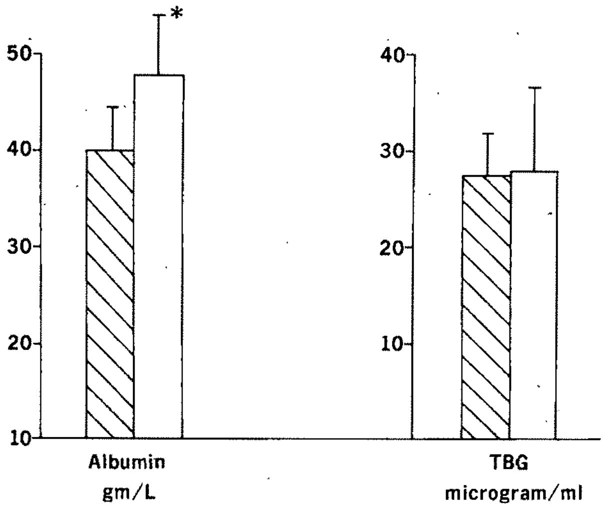

Fig. 2. The mean (±SD) values of serum albumin and serum thyroid-binding globulin *(TBG)* before operation *(hatched)* and after maximum weight loss are shown. A significant difference was observed in the serum albumin level (*p < 0.001) after maximum weight loss.

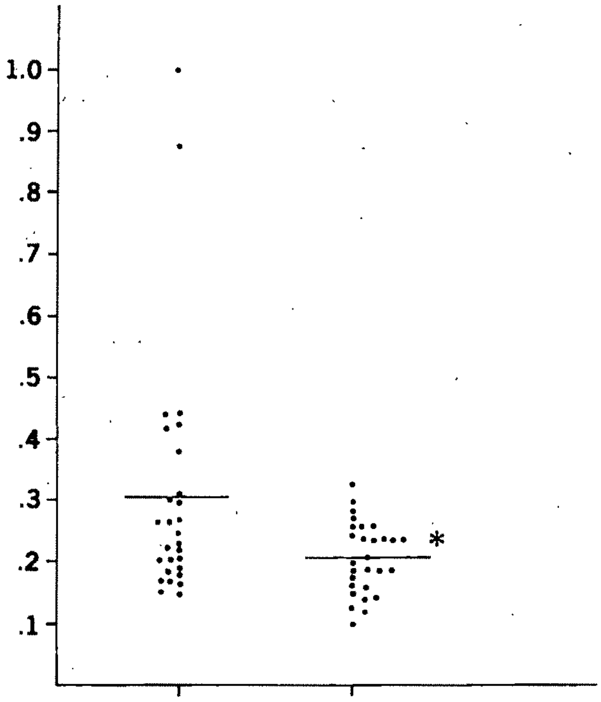

Fig. 3. The individual serum reverse triiodothyronine values before operation *(left)* and after maximum weight loss *(right)*. The mean value for the group after maximum weight loss was significantly lower than the preoperative value. * = $p < 0.018$.

triiodothyronine is the active metabolite responsible for energy expenditure, the decrease in thyroxine to triiodothyronine conversion and the reciprocal increase in thyroxine to reverse triiodothyronine conversion is viewed as an attempt at metabolic conservation. All patients do not consistently demonstrate this reciprocal relationship between serum reverse triiodothyronine and triiodothyronine.[8] In our patients the decreased serum reverse triiodothyronine level with no change in serum triiodothyronine and serum thyroxine levels could be explained by the following: (1) a decreased production rate of reverse triiodothyronine from thyroxine, (2) an increased clearance rate of reverse triiodothyronine, (3) an increased proportion of thyroxine being converted to another metabolite such as tetraiodothyroacetic acid.

Grant et al.[9] described an increase in tetraiodothyroacetic acid production in fasting patients with hepatic cirrhosis. A similar situation may exist in our subjects in that other metabolites of thyroxine that we did not measure may be produced when the reverse triiodothyronine is decreased.

Grant et al.[9] demonstrated that acute weight loss on a semistarvation formula diet (containing a mixture of protein and carbohydrates) resulted in normal serum reverse triiodothyronine values but low serum triiodothyronine levels. It has been shown that the addition of glucose to a starvation diet will also normalize low serum triiodothyronine levels.[10] There is then a sensitive response in thyroid hormones to dietary constituents. The diet which anorexic patients follow is variable in carbohydrate and glucose content and, in order to achieve such rapid weight loss, it often is much lower in calories than 750 kcal per day. Although hypoalbuminemia may result in severe cases of protein malnutrition such as Kwashiorkor, this is rarely seen in subjects with anorexia nervosa. Thus the changes in thyroxine metabolism need not be accompanied by changes in serum albumin. The observed increase in serum albumin after weight loss was unexpected. We plan to use more specific techniques to measure albumin in order to assess the importance of this observation. Our population of morbidly obese women ate a mixed protein-carbohydrate diet with a much lower caloric count than that prior to gastric plication but far in excess of a true fasting state. Moreover, the majority of our obese women were still above their ideal weight even at their point of maximum weight loss. This is quite different from women with anorexia nervosa who are 20% to 30% below ideal weight when thyroid hormone changes are noted. In anorexia nervosa, the weight loss may occur over a very brief period of time while our obese subjects demonstrated their weight loss over a more prolonged time interval of 1 to 2 years.

As illustrated in Fig. 3, although the mean reverse triiodothyronine for subjects before operation was higher than the value after weight loss, only six subjects had preoperative reverse triiodothyronine levels higher than the highest value after weight loss. The decrease in reverse triiodothyronine may be explained largely by the decrease in these six patients. Thus two subgroups may exist in an obese population with respect to serum reverse triiodothyronine levels. Regardless, the expected increased reverse triiodothyronine, seen in anorexia nervosa, certainly was not observed in these obese subjects.

Although women with anorexia nervosa and weight loss after gastric plication are similar in that they experience profound weight loss, the serum reverse triiodothyronine value moves in opposite directions. Perhaps the degree of adiposity per se rather than caloric intake or amount of weight may be the important variable influencing serum reverse triiodothyronine metabolism.

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Suppressor cells in human decidua

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Decidual suppressor cells have been found in the murine system. These cells are absent at the implantation sites of interspecies mouse embryos which become infiltrated by maternal cytotoxic cells. Suppression is also deficient at the sites of the spontaneous resorption of fetuses in allomated intraspecies pregnancies. This study was carried out to determine whether similar suppressor cells were present in the decidua during successful human allopregnancies. Decidua was obtained from gestations of 13 to 15 weeks and from term gestations, and the lymphocytes were tested for their ability to suppress the response of their peripheral blood lymphocytes to concanavalin A. Eight of eight 13- to 15-week decidual lymphocytes proved to be suppressive. At term seven of twelve lymphocyte preparations at a lower concentration of cells and six of seven at a higher concentration showed suppression. Suppressor cells appear to be present in human decidua and may play a role in preventing maternal immunologic attack on the allogenic embryo, thereby preventing spontaneous abortion. (Am J Obstet Gynecol 1985;151:267-70.)

Key words: Suppressor cells, human decidua, spontaneous abortion

The mammalian fetus expresses paternal antigens against which the maternal immune system can and often does respond, but the fetus is not invariably rejected.[1-4] The fetus may therefore be viewed as a type of allograft that survives in an immunologically hostile host. This survival has been shown to be determined by the properties of the tissue at the interface between the fetus and the mother. For example, transplantation of one of the intrauterine fetuses of an allopregnant

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Table I. Typical results

	Counts per minute			
	*Actual**	*Expected*	*Actual/ expected (%)*	*% suppression*
Peripheral blood lymphocytes 1 × 10^5	70,987 ± 5671	24,222	293	Nil
Peripheral blood lymphocytes 5 × 10^4	12,111 ± 155			
Decidual lymphocytes 1 × 10^5	49,402 ± 2301	60,328	82	18
Decidual lymphocytes 5 × 10^4	30,164 ± 2987			
Peripheral blood lymphocytes + decidual lymphocytes 1 × 10^5 each	44,645 ± 3481	120,389	37	63
Peripheral blood lymphocytes + decidual lymphocytes 5 × 10^5 each	29,503 ± 3253	42,275	70	30

*Standard errors of the mean are shown.

rat to her thigh muscle resulted in a rejection of the tissue by the mother without harm to the remaining intrauterine fetuses.[5] Paternal tumors that were grafted at a systemic site in allopregnant mice were rejected without difficulty.[6] In addition, allogeneic blastocysts placed under the kidney capsule in previously immunized mice were rejected but became implanted and developed normally when placed within the hormonally prepared uterus.[7, 8] These data indicate that local factors within the uterus play a key role in preventing destruction of the conceptus in early pregnancy.

Allografts placed on hormonally induced decidua or on the decidua of pregnant animals become implanted and survive for a much longer period of time than allografts placed at nonuterine sites.[9-12] This suggests that decidua may enhance the survival of such allografts as the fetus. The existence of a localized nonspecific (suppressor) mechanism in the decidua to account for fetal survival would explain how potent immunosuppression could occur in pregnant individuals without rendering them unduly susceptible to infections as would be predicted if a potent nonspecific suppressor mechanism acted systemically. Cells with nonspecific suppressor activity have recently been found in murine decidua.[13-15] These cells as well as those in the lymph nodes draining the pregnant uterus act to impair the ability of T cells to develop into cytotoxic effectors.[16-19] Soluble suppressor activity has also been obtained from cultures of murine decidual suppressor cells. Some recent studies suggest that a soluble suppressor factor is also obtainable from human decidua, but the cell of origin has not yet been determined.[20]

The purpose of this study is to test lymphocytes isolated from human decidua for nonspecific suppressive activity. The result is consistent with the presence of local immunologic suppression that may protect the early human conceptus from maternal rejection.

Material and methods

Preparation of peripheral blood lymphocytes. Following the signing of informed consent forms, 10 ml of venous blood was drawn into heparinized tubes from healthy pregnant women just prior to their operation. The blood was diluted 1:2 in phosphate-buffered saline solution. The lymphocytes were separated following density centrifugation with Isopaque-Ficoll and washed twice with phosphate-buffered saline and finally with α-minimal essential medium. This medium was supplemented with 10% fetal bovine serum, HEPES 20 mmol/L, penicillin 100 μg/ml, streptomycin 100 μg/ml, sodium bicarbonate, and L-glutamine 2.92 mg/100 ml. Cells were suspended in this medium at a concentration of 2 × 10^6/ml.

Preparation of decidual lymphocytes. Decidua was obtained by curettage following dilatation and evacuation of pregnancies of 13 to 15 weeks' gestation. In patients at term the decidua was removed manually with a gauze at elective cesarean sections. The material was pressed gently through a 60-mesh stainless steel sieve, and the lymphocytes were isolated by density centrifugation as described above and suspended at a concentration of 1 × 10^6/ml.

Trypan blue staining showed the viability of the lymphocytes from both decidua and peripheral blood to be 95% to 100%.

Preparation of cultures. Ninety-six–well, round-bottomed plates (Linbro; Flow Laboratories, Inc., Mississauga, Ontario) were used for lymphocyte cultures. The peripheral blood lymphocytes and decidual lymphocytes were added to eight replicate wells as shown in Table I. Concanavalin A, at a final concentration of 10 μg/ml, was used as the mitogenic stimulant. The plates were incubated in a carbon dioxide incubator (Forma Scientific) with a humidified atmosphere of 6% to 7% carbon dioxide in air at 37° C for 48 hours. ^{3}H-thymidine (New England Nuclear) in medium with a final concentration of 2.5 × 10^{-3} μCi/ml was added 16 to 18 hours before harvest.

Harvesting and scintillation counting. The cells in the cultures were washed onto glass fiber filters (Skatron Inc., Sterling, Virginia) by means of a multiple-cell culture harvester (Skatron Titertek cell harvester, Skatron Inc., Sterling, Virginia). Filter sheets were dried at 60° C for an hour. Each filter disk, corresponding to one microculture well, was placed into a plastic scintillation vial; 5 ml of scintillation fluid (4 liters of toluene, 300 mg of 1,4-bis[2(5-phenyloxazolyl)]benzene; phenyloxazolylphenyl-oxazolylphenyl, and 24 gm of 2,5-diphenyloxazole) was then added to each vial. The radioactivity was measured in a liquid scintillation counter (Beckman Model No. LS-233) for 1 minute.

Results

The degree of ^{3}H-thymidine uptake (counts per minute [CPM] with concanavalin A minus counts per minute in medium alone) was calculated, and the mean and SEM of the eight replications were determined for each group. The level of suppressive activity exerted by decidual cells on peripheral blood lymphocytes (PBL) was calculated by means of the following formula:

$$\% \text{ suppression} = \frac{\text{Predicted CPM} - \text{Actual CPM}}{\text{Predicted CPM}} \times 100$$

Predicted counts were calculated as the sum of the respective actual counts, e.g., PBL $10^5_{\text{(predicted)}}$ + PBL $5 \times 10^4_{\text{(actual)}}$ + PBL $5 \times 10^4_{\text{(actual)}}$.

In order to carry out these calculations, a simplifying assumption is made so that the respective actual counts can be added to obtain the predicted counts. This is done in order to provide a number that reflects the effects that are observed.

Table I shows a representative example of the type of results that were obtained. It is clear from this table that fewer counts were incorporated in co-cultures with use of decidual lymphocytes and peripheral blood lymphocytes than when peripheral blood lymphocytes were used with peripheral blood lymphocytes. This implies that the suppressor activity was localized to decidual lymphocytes. Fig. 1, *B*, demonstrates a dose-response effect that further provides more meaning to the suppression calculation.

Fig. 1 shows the level of suppression obtained in a series of experiments done at two gestational age periods during pregnancy. Also shown are the means and one standard deviation of the mean. At the gestation time of 13 to 15 weeks (denoted by *A*) eight of eight samples of decidual cells showed suppressive activity. With these experiments the cell yield was often poor and it was only possible to test 5×10^4 decidual cells/well in culture. At the term gestational period (denoted by *B*), seven of 12 samples of decidua showed suppressive activity by means of a concentration of 5×10^4 cells/well. With the higher concentration of 1×10^5 cells/well, six of seven samples of decidua showed suppressive activity. In this latter group, three of seven showed enhancement activity by means of the lower concentration of cells; however, when the concentration was doubled, all three showed suppressive activity.

Suppressive activity appeared to be lower with the term gestation decidual samples as compared to the 13 to 15 weeks' gestation samples.

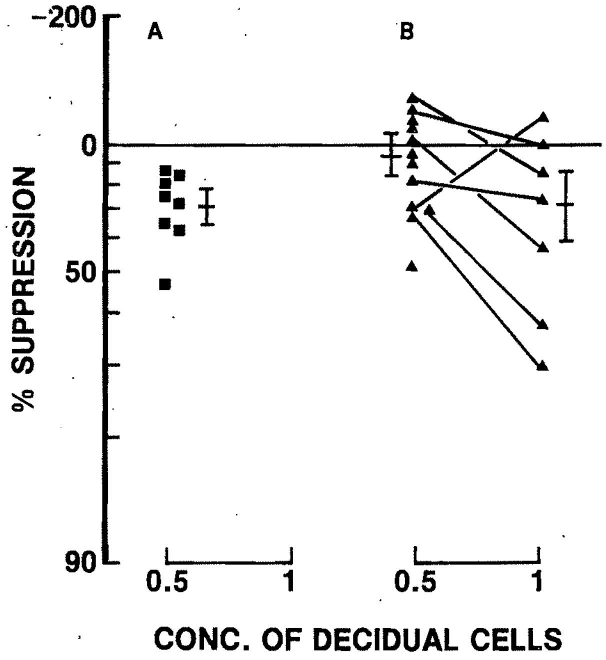

Fig. 1. Suppressive activity of decidual cells.

Comment

As in the murine system, human decidual lymphocytes clearly may exert suppressive activity on maternal peripheral blood lymphocyte reactivity. The effect is nonspecific, since it affects mitogenic stimulation. A differential analysis of the decidual lymphocytes obtained was not carried out. However, using a velocity sedimentation separation technique, we have found that the cell responsible for most of the suppression seen is a very small lymphocyte (unpublished observations). These decidual suppressor cells are found to be present in the first trimester and are also seen at term. One can therefore surmise that these cells may be present throughout normal pregnancies. In the murine system, suppressive activity is seen in the decidual cells prior to implantation,[14] and it is possible that such activity is also present in human decidua. The impracticality of obtaining human material this early in pregnancy makes it difficult to test this theory. It is not known whether this generation of suppressor cell activity in the mouse is triggered by semen or the hormonal changes associated with pregnancy or by a yet unidentified factor. Some information suggests that the decidual suppressor cell may be of relevance to the success of pregnancy. For example, experiments in mice have shown the apparent absence of suppressor cells in uterine-draining lymph nodes[18, 21] and recently in decidua[22, 23] (also D. A. Clark, unpublished observations) in inbred strains of *Mus musculus* that resorb their fetuses. Furthermore, decidual suppressor cells were deficient at the implantation sites of live xenogeneic *Mus caroli* conceptuses that eventually underwent necrosis, resorption, and infiltration by cytotoxic cells in *Mus musculus* uteri.[24] Failure of the xenogeneic trophoblast that bears xenoantigens[25] to recruit local suppressor cells could explain the apparent rejection of the *Mus caroli* xenoembryos.[14] All these studies emphasize the importance of nonspecific suppressor cells, which are recruited to the decidua and thereby prevent a pregnancy from being aborted by a maternal immunologic attack.

The decidual suppressor cell in the murine system is a small lymphocyte with a diameter of 7 μm, has a sedimentation rate of 3 mm/hr, lacks surface markers of T cells, elaborates a soluble factor or factors that suppress cytotoxic T lymphocyte generation, and possesses cytoplasmic granules.[14] Studies to characterize this decidual suppressor cell in the human system are currently under way. Preliminary data suggest that it is also a small lymphocyte with scant cytoplasm in which small granules are present.

Fig. 1 shows a dose-response phenomenon in the term decidua samples, i.e., as the cell concentration is increased, the level of suppression obtained by the decidual lymphocytes is increased. This phenomenon was seen in six of seven samples tested. This implies that there is a certain threshold concentration of decidual lymphocytes below which little or no suppressive activity can be seen. This is probably the result of saturation of helper cells at higher concentrations, which allows suppressor cell activity to be detected.

Fig. 1 also shows an apparent decline in decidual lymphocyte suppressor activity as term approaches in comparison to the activity in early pregnancy. This effect has been described as being present in the murine system, in which a peak level of suppressive activity occurs just prior to implantation followed by a progressive decline toward term.[14]

The challenge that awaits is to identify the stimulus for suppressor cell recruitment to the decidua. This information may then assist in providing treatment for women who have habitual abortion and also perhaps in the normal infertile couple. It is also a challenge to correlate the presence or absence of human decidual suppressor cells with the ultimate outcome of the pregnancy.

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Functional zonation of the midgestation human fetal adrenal cortex: Fetal versus definitive zone use of progesterone for cortisol synthesis

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Studies of human fetal adrenal function and its control have revolved mainly around the remarkable capacity of the unique fetal zone of this gland to elaborate dehydroepiandrosterone sulfate. Another important function of the fetal adrenal, however, is its production of cortisol. Because the human fetal adrenal is deficient in 3β-hydroxysteroid dehydrogenase activity, cortisol has been thought to be formed from circulating progesterone. To further investigate this hypothesis, cortisol production by separated fetal and definitive zones of the midgestation human fetal adrenal in organ culture has been examined in the absence and presence of varying concentrations of progesterone and adrenocorticotropic hormone. Cortisol was measured by radioimmunoassay. In the absence of progesterone, cortisol production by both zones increased gradually over time in culture in response to adrenocorticotropic hormone. In the presence of progesterone, cortisol production by the definitive zone was unchanged. In contrast, the response of the fetal zone to progesterone was immediate: cortisol production increased significantly and remained high throughout the culture period. These results suggest a greater capacity of the fetal zone to utilize progesterone for cortisol production and are consistent with morphologic evidence that the active zone of the midgestation human fetal adrenal is the fetal zone, possessing not only the enzyme activity necessary for dehydroepiandrosterone sulfate production but, except for 3β-hydroxysteroid dehydrogenase, that for cortisol production as well. (AM J OBSTET GYNECOL 1985;151:271-7.)

Key words: Fetal adrenal cortex, progesterone, cortisol synthesis

The human fetal adrenal is 15 to 20 times larger than the adult gland, relative to body weight. This increased size is due to the presence during fetal life of a distinctive fetal zone which constitutes approximately 80% of the gland. Much attention has been focused on the remarkable capacity of the fetal zone to elaborate large quantities of dehydroepiandrosterone sulfate, which serves as precursor for placental estrogen production. The fetal adrenal, however, also produces cortisol, which appears to play an important role in the induction and maturation of a number of fetal enzyme systems in the latter stages of gestation and is involved in the onset of parturition in at least some animal species.

Cord serum cortisol levels have been shown in the human to rise as pregnancy progresses, especially toward term.[1] Since the human fetal adrenal is deficient in 3β-hydroxysteroid dehydrogenase isomerase activity,[2] it has generally been assumed that cortisol is formed from the increasing concentrations of circulating progesterone throughout gestation. Fetal plasma levels of progesterone are, in fact, several times higher than maternal levels.[3] The isolation of ^{14}C cortisol from the adrenals of previable fetuses perfused with ^{14}C progesterone[4] supports this view, as do the results of a number of in vitro studies in which incorporation of labeled progesterone into cortisol by fetal adrenal minces,[5] slices,[6] and explant cultures[7] was demonstrated.

In an earlier attempt to determine whether a corticotropic factor was produced in vitro by placental tissue, medium from homologous placental monolayer cultures was added to explant cultures of the separated fetal and definitive zones of human fetal adrenals. We noted repeatedly that this placental medium enhanced cortisol production by the fetal but not the definitive zone.[8] These results could be mimicked by adding to

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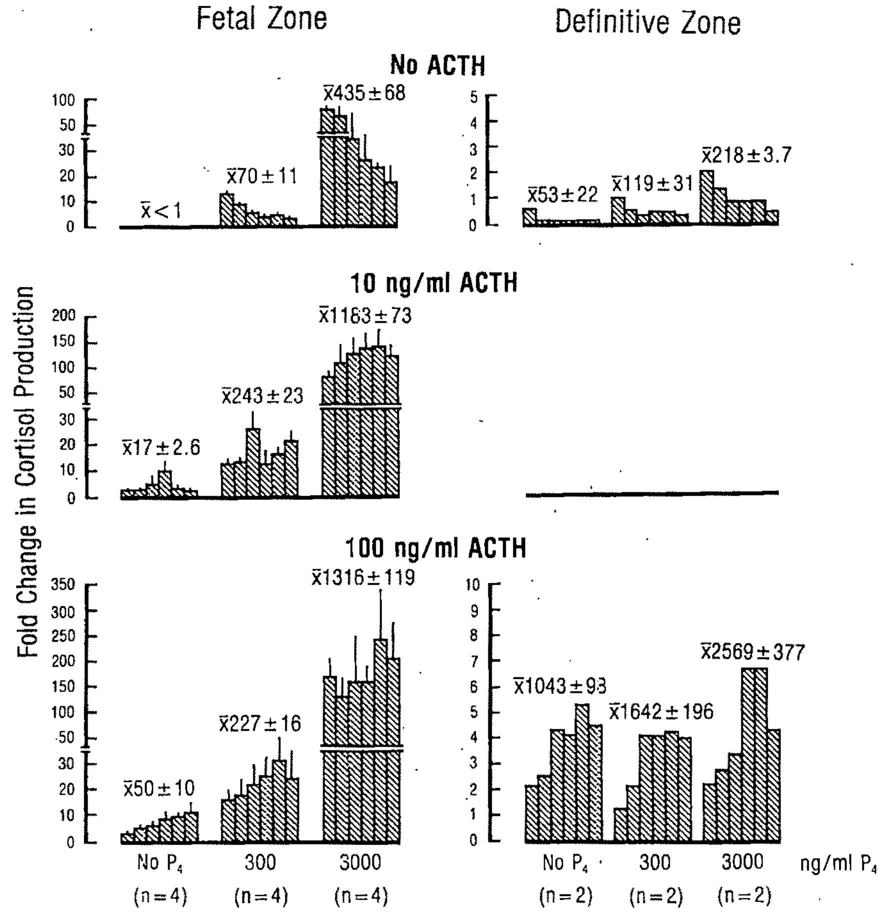

Fig. 1. Mean fold change in cortisol production (from basal) on days 2 to 7 in culture by fetal and definitive zone explants (17½ weeks of fetal age) with or without added adrenocorticotropic hormone *(ACTH)* and/or progesterone *(P_4)*. *Bars* represent mean fold change ± SE for *fetal zone* (n = 4) and mean for *definitive zone* (n = 2). Basal cortisol output was the production during the first 24 hours in culture prior to addition of either adrenocorticotropic hormone or progesterone. *X* numerical values above each set of *bars* represent the mean daily production on days 2 to 7 in culture, expressed as ng/grid/24 hr, mean ± SE. Data were analyzed by factorial two-way analysis of variance: the overall effect of adrenocorticotropic hormone on cortisol production by both the fetal and definitive zones was significant at $p < 0.0001$. The overall effect of progesterone was significant at $p < 0.0001$ in the fetal zone but only $p < 0.05$ on the definitive zone.

the adrenal cultures concentrations of progesterone equivalent to those produced by the placental cells (300 ng/ml).[8] The amount of progesterone used in these studies, however, although greater than the amount of endogenous cortisol produced by the fetal zone, was considerably lower than the levels of cortisol produced by the definitive zone. Thus a contribution of the added progesterone to cortisol output by the definitive zone cultures would not have been readily apparent.

We have therefore examined cortisol production by explant cultures of the separated zones of the midgestation human fetal adrenal in the presence of 300 and 3000 ng/ml of progesterone and have compared the relative capacity of the two zones to utilize this precursor to form cortisol.

Material and methods

Ham's F-10 powdered culture medium and heat-inactivated fetal bovine serum were obtained from Grand Island Biological Company (Burlington, Ontario, Canada), amphotericin B (Fungizone) from Squibb Canada, Inc. (Montreal, Quebec, Canada), penicillin G (Crystapen) from Glaxo Laboratories (Montreal, Quebec, Canada), gentamicin (Garamycin) from Schering (Pointe Claire, Quebec, Canada), and adrenocorticotropic hormone (Duracton, a long-acting preparation of porcine adrenocorticotropic hormone containing carboxymethyl cellulose) from Nordic Laboratories Inc. (Laval, Quebec, Canada). Quartered plastic culture dishes were purchased from Falcon Plastics (Los Angeles, California) and 1.5 cm² culture grids were cut from stainless steel mesh (40 mesh) obtained from Johnson Wires Division, JWI Ltd. (Montreal, Quebec, Canada). Tritium-labeled steroids were purchased from New England Nuclear Canada Ltd. (Lachine, Quebec, Canada) and unlabeled steroids from Steraloids, Inc. (Wilton, New Hampshire). Antisera to cortisol and dehydroepiandrosterone sulfate were obtained from Endocrine Sciences (Tarzana, California).

Adrenal cultures. Fetal adrenals were obtained at the time of therapeutic abortion by vacuum extraction or curettage at gestational ages ranging from 11 to 18 weeks. Ethics approval for the use of human material was obtained from the Ethics Committee of the Montreal Children's Hospital and the Ethics, Research, and Professional Services Committees of Hôpital Notre Dame.

The methodology for separating the adrenal zones and for establishing the adrenal cultures has been described previously.[9] Briefly, adrenals were dissected clean of adhering tissue and the capsule carefully stripped from the central core of the gland. Histologic examination has revealed that following this procedure most of the definitive zone tissue remains attached to the connective tissue capsule and that this tissue is virtually free of fetal zone cells. The fetal zone tissue, on the other hand, does retain some ($\leq$5%) definitive zone cells.[9] Each zone was then finely minced with scissors and the resulting pieces of tissue placed on curved stainless steel grids in quartered Petri dishes containing 2 ml of culture medium (Ham's F-10 supplemented with 10% fetal bovine serum and antibiotics: 5 μg/ml of amphotericin B, 200 IU/ml of penicillin G and 40 μg/ml of gentamicin). Eight explants (equivalent to about 3 mg of the smaller adrenals and ranging to about 7 mg of the larger glands) of fetal zone tissue and six explants (3 mg of the smaller glands up to 5 mg of the larger glands) of definitive zone tissue were placed on each grid. Because each adrenal yielded much less definitive zone, fewer grids of this tissue could be set up in each experiment.

The cultures were kept at 37° C in a humidified atmosphere of 5% carbon dioxide in air. The medium was changed daily. Progesterone and adrenocorticotropic hormone were added only after the first 24-hour collection. Thus the cortisol measured in the day 1 medium represents the basal, unstimulated cortisol output by each grid of tissue. Progesterone dissolved in ethanol was added to sterile medium bottles, the ethanol was evaporated under nitrogen, and the steroid redissolved in an appropriate volume of culture medium. Solubility of the progesterone in the culture medium was verified with the use of labeled progesterone and by radioimmunoassay of progesterone in the medium; complete solution of even the higher concentration was easily achieved, caused presumably by binding to fetal bovine serum proteins. Adrenocorticotropic hormone was diluted in sterile saline solution and 10 μl were added daily to each petri quadrant where indicated. The final concentrations of 5, 10, and 100 ng/ml of medium (0.5, 1, and 10 IU/ml) were based on previous observations of those doses that provided maintenance and maximal steroidogenic responses in the adrenal explant culture system.

Steroid determinations. Media collected every 24 hours during the culture period were kept frozen until processed for determination of cortisol and dehydroepiandrosterone sulfate. Dehydroepiandrosterone sulfate was assayed by radioimmunoassay directly on the culture media without prior extraction or chromatography. Antiserum to dehydroepiandrosterone sulfate (Endocrine Sciences) was raised in rabbits against dehydroepiandrosterone sulfate 17-oxime-BSA; its cross-reactivity was <0.01% with dehydroepiandrosterone and 17α-hydroxyprogesterone, 0.03% with androstenedione and androsterone and 0.13% with androsterone sulfate. Similar dehydroepiandrosterone sulfate values were obtained with and without extraction and chromatography.

In earlier experiments (represented in Fig. 2) cortisol was measured by radioimmunoassay following protein precipitation, methylene chloride extraction, and paper chromatography as described previously.[9] In later experiments (represented in Fig. 1) cortisol was determined directly on the culture media without prior extraction or chromatography. Antiserum to cortisol (Endocrine Sciences) was raised in rabbits with the use of a cortisol-3-oxime-BSA conjugate. The cross-reactivity of this cortisol antiserum was 0.015% with corticosterone, 3.3% with 11-deoxycortisol, 7.5% with 21-deoxycortisol, <0.01% with progesterone, 0.23% with 17α-hydroxyprogesterone, and <0.01% with 20α- and 20β-dihydroprogesterone. Unchromatographed cortisol values were 15% to 20% higher than chromatographed values. Results are expressed as mean fold change in cortisol production over basal (no adrenocorticotropic hormone, no progesterone, day 1) production, on days 2 to 7 (Fig. 1) or 2 to 6 (Fig. 2) in culture, ±SE where $n = 4$ grids; the numbers above the bars represent the mean daily cortisol production in nanograms per grid per 24 hours ±SE, from day 2 to the end of the culture period. Steroid production has not been expressed per weight of tissue for two reasons: first, with the larger explants of fetal zone tissue, we feel that it is difficult to assess what proportion of the explant has contributed to the amount of hormone released into the medium; second, because a large proportion of the definitive zone explants consists of capsular tissue, weight would not necessarily rep-

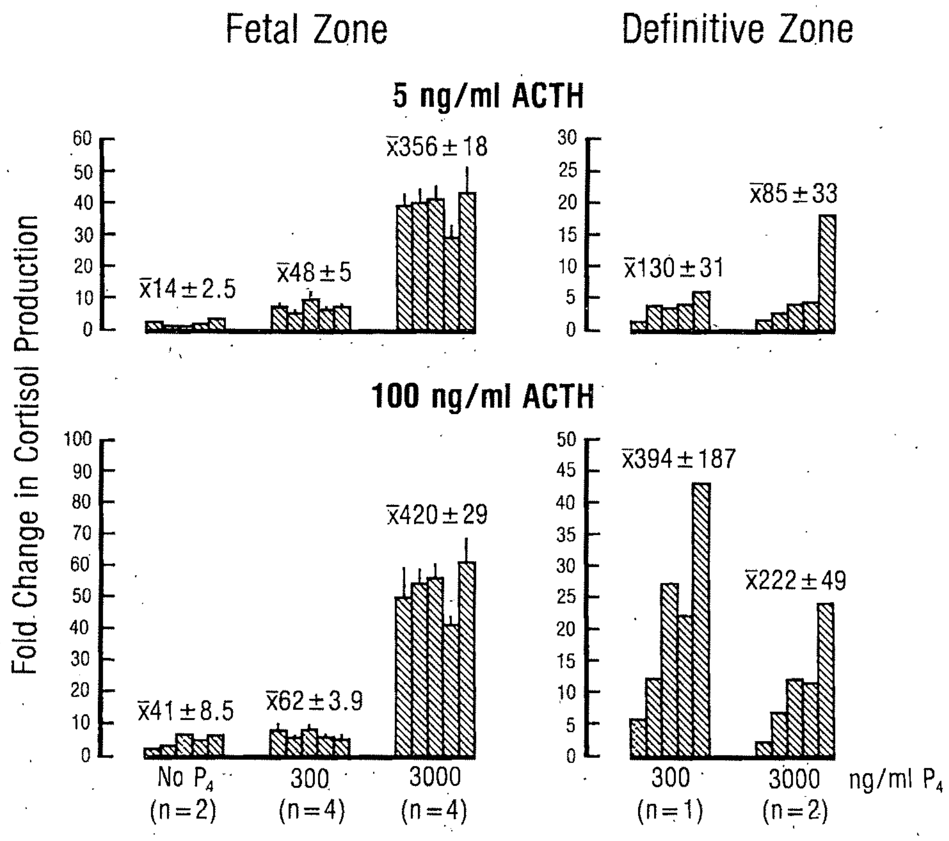

Fig. 2. Mean fold change in cortisol production (from basal) on days 2 to 6 in culture by fetal and definitive zone explants (15½ weeks fetal age) in response to adrenocorticotropic hormone *(ACTH)* and different concentrations of progesterone *(P_4)*. *Bars* represent mean fold change ± SE where n = 4. Basal cortisol output was the production of each grid during the first 24 hours in culture, prior to addition of either adrenocorticotropic hormone or progesterone. *X* numerical values above each set of *bars* represent the mean daily production on days 2 to 6 in culture, expressed in ng/grid/24 hr, mean ± SE. Data were analyzed by factorial two-way analysis of variance: the overall effect of adrenocorticotropic hormone on cortisol production was significant at $p < 0.001$ in the *fetal zone* and $p < 0.03$ in the *definitive zone*. The overall effect of progesterone was significant at $p < 0.0001$ in the fetal zone and was not significant in the definitive zone.

resent the actual amount of definitive zone tissue in each explant.

Data were analyzed by factorial two-way analysis of variance.

Results

The results of two of a series of 10 experiments are presented in Figs. 1 and 2. These experiments were chosen because they represent the range of cortisol production (Fig. 1, high; Fig. 2, low) as well as the response to two doses of progesterone. These results show the effect of 300 and 3000 ng of progesterone and/or 5, 10, and 100 ng of adrenocorticotropic hormone per milliliter of culture medium on cortisol production by fetal and definitive zone explants over several days in culture. As we have reported previously,[8, 9] cortisol output by the definitive zone was greater than that by the fetal zone, while dehydroepiandrosterone sulfate output by the fetal zone was greater than that by the definitive zone. In both of these experiments, the basal dehydroepiandrosterone sulfate/cortisol ratio on day 1 was 10 or less in the definitive zone media and 700 or more in the fetal zone media (data not shown).

In the absence of adrenocorticotropic hormone (Fig. 1, *upper panel*), and without added progesterone, cortisol production falls to very low (definitive zone) or undetectable (fetal zone) levels by the third day in culture. Upon addition of 300 and 3000 ng/ml progesterone in the absence of adrenocorticotropic hormone (Fig. 1, *upper panel*), there was an immediate (day 2) dose-response–related increase in cortisol output by the fetal zone explants; however, this response was not maintained over the culture period under these conditions, i.e., when adrenocorticotropic hormone was not present in the culture medium. The definitive zone cortisol output increased somewhat when progesterone was present, but the response was never as pronounced as that of the fetal zone and, again, was not maintained over the culture period. This difference in response is even more striking when one notes the expanded scale of the fold change by the fetal zone.

In the presence of adrenocorticotropic hormone, but in the absence of progesterone (Fig. 1, *middle* and *lower panels*, and Fig. 2), the output of cortisol by both the fetal and definitive zone cultures increased in a stepwise fashion, reaching a maximum on day 4 or 5. It may be seen in both Figs. 1 and 2 that the fetal zone response to adrenocorticotropic hormone was dose dependent. Limited availability of tissue precluded testing the dose response to adrenocorticotropic hormone by definitive zone explants in these studies, but increased cortisol output in response to increasing doses of adrenocorticotropic hormone by both zones has been observed in a number of previous studies.[9, 10]

Cortisol output in response to 300 and 3000 ng/ml of progesterone by fetal zone tissue was similar whether in the presence or absence of adrenocorticotropic hormone, i.e., there was an immediate (day 2) maximal increase. In the presence of adrenocorticotropic hormone, however, this response was maintained throughout the culture period. Furthermore, the increase in cortisol output was directly proportional to the amount of progesterone added, and this dose-related response to progesterone was not affected by increased concentrations of adrenocorticotropic hormone.

On the other hand, the pattern of cortisol output by adrenocorticotropic hormone–stimulated definitive zone cultures was similar in the presence or absence of added progesterone, i.e., there was a stepwise increase to a maximum on day 4 or 5 and there was no dose response to progesterone. It should be pointed out, however, that the mean daily cortisol output (1043 ng/grid/24 hr) by adrenocorticotropic hormone–stimulated definitive zone in the absence of progesterone was higher than the 300 ng/ml (600 ng/grid) progesterone concentration added to the cultures. Thus a notable response to the added progesterone would only be obvious at the 3000 ng/ml (6000 ng/grid) concentration.

Basal (day 1, unstimulated) dehydroepiandrosterone sulfate production was 1335 and 195 ng/grid/24 hr by the definitive zone cultures and 7900 and 5685 ng/grid/24 hr by the fetal zone in experiments 1 and 2, respectively. Dehydroepiandrosterone sulfate production by both zones decreased with time in culture when adrenocorticotropic hormone was not added to the cultures. In the presence of adrenocorticotropic hormone, dehydroepiandrosterone sulfate production by the fetal zone explants was maintained and that of the definitive zone stimulated as previously reported.[9] Progesterone had no effect on dehydroepiandrosterone sulfate output by either zone (data not shown).

Comment

These findings are further indication of a distinct functional zonation of the human fetal adrenal, in this case with respect to cortisol production. It has been well documented that the fetal zone is deficient in 3β-HSD activity and as a result produces large amounts of Δ^5 steroids, particularly dehydroepiandrosterone sulfate.[11] The present series of studies, of which the results in Figs. 1 and 2 are representative, indicate apparent differences in the ability of the fetal and definitive zones to use progesterone as a substrate for cortisol production. The fetal zone readily used progesterone to form large amounts of cortisol, seemingly independently of adrenocorticotropic hormone, at least early in the culture period. On the other hand, the pattern of cortisol production by the adrenocorticotropic hormone–stimulated definitive zone was similar whether or not progesterone was present. Response to adrenocorticotropic hormone was slow, in that maximal cortisol output was achieved only after several days' exposure to adrenocorticotropic hormone.

We have previously reported that fetal adrenal explants fail to maintain the integrity of their cellular structure when cultured in the absence of adrenocorticotropic hormone and that there is a correlation between steroidogenesis and morphologic integrity.[8, 9] In the present study, decreasing viability of the cultures in the absence of adrenocorticotropic hormone undoubtedly accounts for the fall-off in steroid output by both zones with time in culture, even in the presence of progesterone.

A stimulatory effect of progesterone on cortisol production has been described by Fujieda et al.[12] in monolayer cultures and by Voutilainen et al.[13] in explant cultures of whole fetal adrenals. Fujieda et al.[12] also noted that even high concentrations of progesterone had no effect on dehydroepiandrosterone sulfate production. Voutilainen and Kahri,[14] however, reported that co-culturing fetal adrenal and placental explants resulted in a suppression of adrenal cortisol production. We have noted (unpublished observations) that placental explant cultures produced very low levels of progesterone as compared to placental monolayer cultures, a finding which could at least partly explain the difference between these authors' results and ours.

In order to assess the effect of culture conditions on cortisol output, we have recently used short-term (4-hour) incubations of fresh fetal adrenal minces in Ham's F-10 medium without fetal bovine serum. Once again the fetal zone tissue displayed a much greater capacity to use progesterone to form cortisol than did the definitive zone, thus confirming the results of the culture studies (unpublished observations).

The present findings are in agreement with morphologic observations that at midgestation, the fetal zone is an active steroid-producing tissue whereas the definitive zone is a relatively undifferentiated tissue.[11] The ready use of progesterone by the fetal zone to form cortisol indicates the presence of abundant 17α-, 21-, and 11β-hydroxylase activity in this tissue and suggests that the activity of these enzymes is not greatly influenced by adrenocorticotropic hormone. It has also been demonstrated that the fetal zone is adequately equipped to produce large amounts of dehydroepiandrosterone sulfate from cholesterol derived either from lipoprotein cholesterol or formed de novo by the tissue.[11] Thus the fetal zone would appear to be deficient mainly in 3β-hydroxysteroid dehydrogenase activity, this enzyme either being absent or present in an inhibited state.

The pattern of cortisol synthesis by the definitive zone suggests that exposure to adrenocorticotropic hormone over several days in culture results in an activation of the enzyme systems involved in making cholesterol available for cortisol synthesis, an observation reported by others as well.[11] However, activation of other enzymes such as 3β-hydroxysteroid dehydrogenase may also be involved.[15] The pattern of dehydroepiandrosterone sulfate production by definitive zone cultures may be analogous to that of cortisol. We have noted repeatedly that both dehydroepiandrosterone sulfate and cortisol output increase with time in culture in response to adrenocorticotropic hormone.[8, 9] In contrast, dehydroepiandrosterone sulfate production by fetal zone tissue is maintained at a relatively constant rate under the same conditions. It would appear that, whereas the definitive zone cells develop from an inactive to an active state in response to adrenocorticotropic hormone in culture, the fetal zone cells are highly active from the outset, at least with respect to C_{19} steroid synthesis.

The similar pattern of cortisol production by both zones in response to adrenocorticotropic hormone, in the absence of progesterone, is more difficult to explain. The much lower production rate by the fetal zone suggests that this could be due to the contaminating definitive zone cells that remain attached to the fetal zone following the separation technique.[9] It could also be that 3β-hydroxysteroid dehydrogenase and/or other enzymes are induced in both zones by adrenocorticotropic hormone with time.

Murphy[16] has reported high concentrations of progesterone in both fetal blood and fetal adrenal tissue at midgestation (150 ng/ml in cord arterial, 350 ng/ml in cord venous blood, and 500 ng/gm of adrenal tissue). In a later study Murphy found that when the adrenals were fast frozen in liquid nitrogen to prevent rapid metabolism, the progesterone concentration in the tissue was, in fact, several times higher (personal communication). In view of the present observations, it is tempting to speculate that the fetal zone might have some mechanism for concentrating progesterone within the cell, making it available for cortisol production.

Although the present studies were carried out using midterm fetal adrenals, the results suggest that fetal zone tissue is equipped to supply, from progesterone, the increased amounts of cortisol required by maturing fetal tissues toward the end of gestation. It is of interest to note that fetal blood levels of progesterone at term are two to two and a half times higher than at midterm,[16, 17] a difference similar to that seen in fetal plasma cortisol levels at 36 weeks as compared to 15 to 18 weeks.[1]

It should also be pointed out that although the midterm fetal adrenal does produce cortisol, it is rapidly metabolized to cortisone by 11β-hydroxysteroid dehydrogenase present in virtually all midgestation fetal tissues, and that these tissues are unable to reactivate cortisone.[18] Toward term the fetal tissues no longer inactivate cortisol to cortisone, but their capacity for activating cortisone does not develop until after birth.[18] Since 80% of maternal cortisol is inactivated by placental 11β-hydroxysteroid dehydrogenase during transplacental passage even at term,[16, 18] the late gestation fetus must be self-sufficient with regard to its cortisol supply.

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A more complete list of relevant references will be furnished by the authors upon request.

Effect of naloxone on menopausal flushes, skin temperature, and luteinizing hormone secretion

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The effect of naloxone (1.4 mg/hr for 3 hours) on subjectively experienced menopausal flushes, skin temperature, and luteinizing hormone secretion was investigated in seven women in a double-blind, saline-controlled, crossover study. Naloxone had no effect on the number of subjective flushes, episodic skin temperature elevation, luteinizing hormone pulses, variability of luteinizing hormone secretion, or total luteinizing hormone secretion. This study suggests that a naloxone-sensitive opioid mechanism is not active in modulating luteinizing hormone secretion in the postmenopausal woman and that opioid receptor blockade is not effective in altering the frequency of menopausal flushes. (AM J OBSTET GYNECOL 1985;151:277-80.)

Key words: Naloxone, menopausal flushes

The etiology of menopausal flushes is unclear. Various investigators have noted a temporal relationship between subjectively experienced menopausal flushes, episodic skin temperature elevations, and pulsatile luteinizing hormone (LH) secretion.[1, 2] The rôle of opioids in the pathophysiology of menopausal flushes has been suggested by several investigators.[3, 4] The present study was undertaken to investigate the effect of an infusion of naloxone on subjectively experienced flushes, episodic cutaneous temperature elevations, and pulsatile LH secretion.

Material and methods

Seven postmenopausal women aged 48 to 53 years who complained of frequent distressing flushes and who gave their informed consent served as subjects. They were in good physical health and had received no medication for at least 4 weeks before the study.

Each subject received naloxone hydrochloride (Nar-

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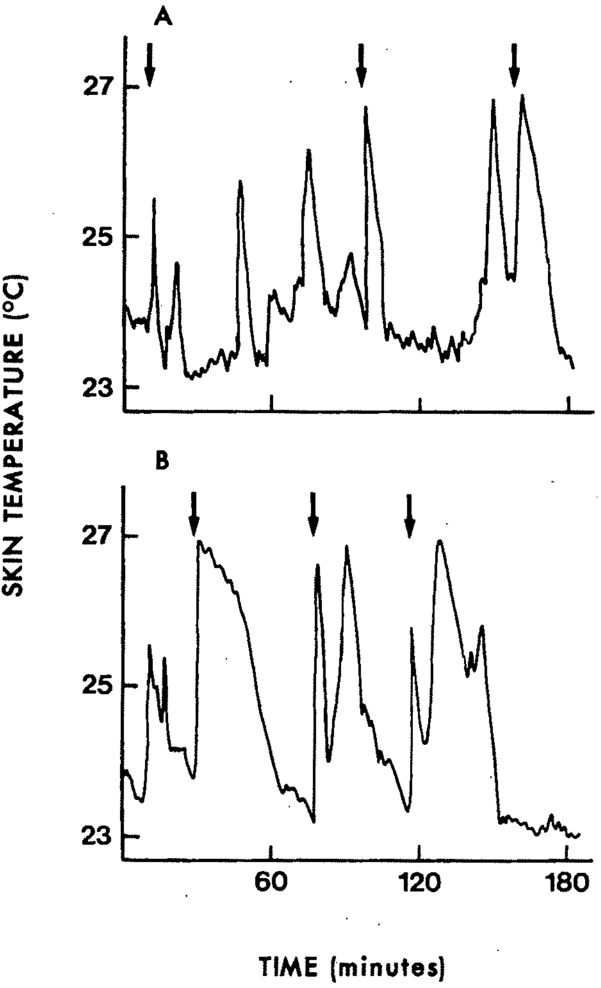

Fig. 1. Changes in skin temperature in a postmenopausal patient after saline solution *(A)* and naloxone infusion *(B)*. The onset of hot flushes is indicated by *arrows*. Note that naloxone had no effect on the number of hot flushes or on the episodic skin temperature elevation.

can. Endo Labs., Inc.) infusion or physiologic saline solution as placebo. This was done in random order and in double-blind fashion. Both tests were conducted under identical environmental conditions and the ambient temperature did not exceed 22° C.

At time zero an intravenous infusion of naloxone (1.4 mg/hr) or physiologic saline solution alone was started. Samples of blood were drawn at 15-minute intervals over a 3-hour period, commencing at time zero for LH determination. Skin temperature was recorded continuously over the dorsum of the proximal phalanx of the third finger of the nondominant hand with a YSI (Yellow Springs Instruments, Yellow Springs, Ohio) banjo surface thermistor (T2625) and telethermometer (T2450) which was connected to a YSI model recorder as previously described.[1, 2, 5] Patients were asked to report on the onset and intensity of each flush. The subjective intensity was rated on a three-point scale, namely, mild, moderate, or severe. The numbers of cutaneous temperature peaks (elevation >1° C above baseline were counted according to the criteria of Meldrum et al.[6] and blinded as to treatment code.

Blood samples were centrifuged and the plasma stored at −20° C until assayed for LH by a standard radioimmunoassay. Samples from each subject from the two test sessions were assayed together to avoid interassay variability. LH peaks were identified using the criteria of Santen and Bardin.[7] Variability in LH secretion was assessed by calculating the coefficient of variation over the 3-hour sampling period. LH secretion was calculated by integrating the area under the curve when the LH concentration was plotted against time.

Data were analyzed by the paired t test and the Pearson product moment correlation coefficient.

Results

In the seven postmenopausal women, 47 menopausal flushes and 68 temperature peaks during saline solution infusion and 40 menopausal flushes and 69 temperature peaks during naloxone infusion were recorded (Fig. 1).

Neither treatment was associated with side effects. There was no significant difference between the number of menopausal flushes during saline solution and naloxone infusion (Table I).

During naloxone infusion compared with saline solution infusion, there was no significant difference in the number of temperature peaks, number of LH pulses, variability in LH secretion, or total LH secretion (Table II).

There was no significant correlation between number of subjective flushes and number of temperature peaks during saline solution ($r = 0.72$, $df = 5$) but these variables were significantly correlated during naloxone ($r = 0.82$, $df = 5$, $p < 0.05$). When data for the two treatments were combined there was a significant correlation between number of temperature peaks and number of subjective flushes ($r = 0.77$, $df = 12$, $p < 0.01$). In some of the subjects there was a striking similarity in the number of flushes and cutaneous temperature peaks. Thus one subject experienced 12 flushes and 13 temperature peaks after saline solution and 16 flushes and 18 temperature peaks after naloxone.

There was no significant correlation between the number of subjective flushes and the LH pulses during saline solution ($r = 0.02$, $df = 5$) or naloxone ($r = 0.43$, $df = 5$) or on examination of the combined data for the two treatments ($r = 0.24$, $df = 5$). There was also no significant correlation between the number of temperature peaks and the number of LH pulses during saline solution ($r = -0.27$, $df = 5$) or naloxone

Table I. The number and intensity of subjective flushes during saline solution and naloxone infusion

Treatment	Menopausal flushes			
	Mild	Moderate	Severe	Total
Saline	2.71 ± 0.99	3.14 ± 1.40	1.00 ± 0.44	6.9 ± 1.2
Naloxone	1.86 ± 0.55	2.85 ± 2.19	1.14 ± 0.51	5.9 ± 1.8
p	NS	NS	NS	NS

Table II. Effect of naloxone on skin temperature and LH secretion

Treatment	No. of temperature peaks*	No. of LH pulses*	LH secretion† (coefficient of variation, %)	LH secretion ($\mu g \cdot min \cdot ml^{-1}$)			
				1st hr	2nd hr	3rd hr	Total
Saline	9.7 ± 0.8	2.71 ± 0.29	19.7 ± 3.8	11.74 ± 1.27	12.56 ± 1.57	11.83 ± 1.47	36.12 ± 4.04
Naloxone	9.9 ± 1.8	3.0 ± 0.38	20.2 ± 2.4	11.31 ± 1.62	12.05 ± 1.07	11.25 ± 1.17	34.61 ± 3.60
p	NS	NS	NS	NS	NS	NS	NS

Data are presented as the mean ± SEM (n = 7).
*Number during the 3-hour period.
†Variation in LH during the 3-hour period.

($r = 0.28$, df = 5) or on examination for the two treatments ($r = 0.14$, df = 12).

Comment

Several investigators have pointed to a temporal relationship between menopausal flushing, cutaneous temperature elevation, and pulsatile LH secretion.[1,2] In the present study the number of subjectively experienced flushes was significantly correlated with the number of cutaneous temperature elevations but not with the number of LH pulses. Although the close temporal relationship between the onset of flushes and the occurrence of pulsatile LH release suggests that they may share a common inciting mechanism within the hypothalamus, a dissociation of these events may occur in other conditions.[5]

Lightman et al.,[4] using a naloxone infusion (1.32 mg/hr during four 90-minute periods) and a saline solution placebo in six women, found a significant decrease in subjective flushes with naloxone; skin temperature was not monitored. In contrast, in the present study, naloxone (1.4 mg/hr during 3 hours) had no effect on the number of subjective flushes or on the number of cutaneous temperature peaks. The reason for this difference is unclear. In both studies the number of flushes experienced during exposure to saline solution infusion was similar. The failure of naloxone to affect skin temperature is in keeping with the findings of Cohen and Coffman[8] that intravenous naloxone has no effect on peripheral vascular flow.

Opiate peptides inhibit LH release in premenopausal women.[9] The opiate antagonist naloxone increases both the frequency and the amplitude of LH pulses in the luteal phase but fails to stimulate LH release in the early proliferative phase or following bilateral oophorectomy.[10, 11] These latter findings suggest that naloxone effects on LH secretion are estrogen dependent. This would explain the failure of naloxone to affect LH secretion in our postmenopausal women in whom plasma estrogen levels are known to be low. A similar lack of effect of naloxone on LH secretion in postmenopausal women has recently been reported by Reid et al.[12]

The lack of effect of naloxone, an opioid receptor blocker, is due to the extremely low endogenous opioid tone in postmenopausal women. However, in contrast to these findings, Lightman et al.[4] noted a decrease in number of LH pulses after naloxone.

The results of the present study confirm the findings of de Fazio et al.[13] that naloxone has no effect on the occurrence of hot flushes, episodic skin temperature elevations, and LH secretion.

In conclusion, our findings suggest that a naloxone-sensitive opioid mechanism is not active in modulating LH secretion in the postmenopausal woman and that opioid receptor blockade is not effective in altering the frequency of menopausal flushes.

We wish to express our appreciation to Nadia Zajac for her technical assistance.

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The variability of fetal breathing movements in normal human fetuses at term

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With the use of a B-scan phase-locked tracking system, 108 observations of fetal breathing were performed on 97 normal antenatal patients at term. One hundred thirty-three recorded segments of fetal breathing movements were obtained. Breath-to-breath variability was analyzed in 79 segments and expressed as a coefficient of variability. The mean coefficient of variability was 28.6% ± 13.2%, and a histogram plot revealed a normal distribution. All fetuses had normal antepartum testing and all Apgar scores and neonatal courses were normal. The potential application of human fetal breathing variability as a test for fetal health is discussed. (Am J Obstet Gynecol 1985;151:280-2.)

Key words: Phase-locked tracking, breath-to-breath variability, fetal health

Various clinical studies have correlated fetal outcome with the presence or absence of breathing and the percentage of time spent breathing.[1-3] In the fetal lamb hypoxia is known to be associated with prolonged apnea.[4] However, because fetal breathing movements are normally intermittent, their absence might not denote fetal compromise. Conversely, the mere presence of fetal breathing movements might not guarantee fetal well-being and observations on sheep have shown that the pattern of breathing, whenever present, is of importance as a diagnostic index of fetal health.[5] Therefore, in humans specific features of fetal breathing such as depth, variability, slope, and frequency might help distinguish healthy from sick fetuses. Quantification of these features has been facilitated by both continuous Doppler ultrasound and pulse-echo tracking systems.[6, 7] With the latter system used to record fetal breathing movements, this study presents our observations of fetal breathing variability (breath-to-breath variability) in normal human fetuses at term.

Material and methods

Consent to study fetal breathing movements was obtained from 97 normal antenatal patients at the Toronto General Hospital between May, 1980, and April, 1982. All pregnancies were uncomplicated and all observations were obtained between 38 and 42 completed weeks of gestation. Recordings were made during a 30-minute period between 8:00 AM and 6:00 PM, in a quiet, darkened room with the patient in a semilateral position. One hundred eight such recordings were obtained in the 97 patients.

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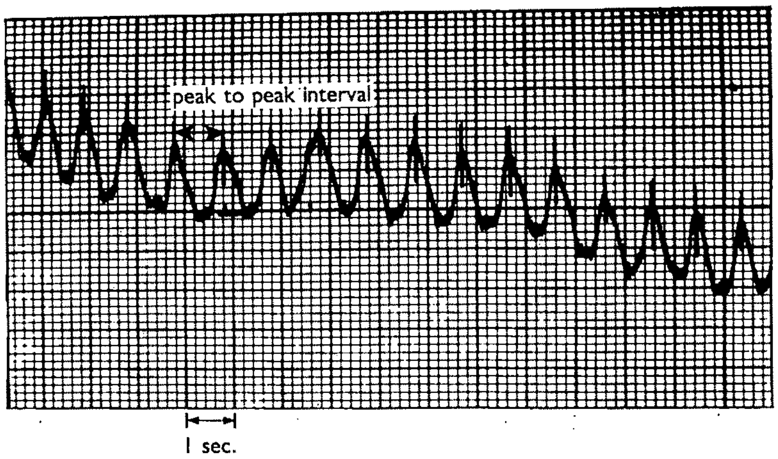

Fig. 1. Chart recording of fetal breathing movements. Paper speed = 5 mm/sec. Vertical sensitivity of 8 mm = 4 mm of fetal movement.

Fetal breathing movements were observed by means of a 3.5 MHz, 64-element, linear crystal array transducer. The instrument used features a resolution measured in micrometers compared to the millimeter resolution of conventional B-scan imaging systems, the capability to record any portion of the echo complex for tracking purposes, and the ability to withstand variations in echo amplitudes without producing artifactual recording.[8] Two intensified dots on the display format correspond to tracker channels and can be positioned on the screen to coincide with the desired image structure. To obtain differential motion information, one is required to look at the dynamic variation of the difference between the two dot positions. The analogue output of each set of tracking channels is recorded on a chart recorder and accurately reflects patterns of activity seen on the video monitor (Fig. 1).[7]

Fetal breathing movements were first identified when the longitudinal scan of the fetus revealed rhythmic chest and abdominal wall movements occurring at intervals of <6 seconds.[9] The intensified dots were then positioned on selected anterior and posterior abdominal wall echoes at a level where the dots moved maximally away from each other creating a well-defined deflection of the needle on the chart recorder. Because the deflection peak (height of inspiration) was usually a brief, clearly defined phase of the respiratory cycle, peak-to-peak intervals were measured in the calculation of rate, mean breath-to-breath interval, variance, and coefficient of variability (coefficient of variability = SD of breath-to-breath interval/mean of breath-to-breath interval × 100%).

In each instance a 20-minute nonstress test was performed immediately prior to the 30-minute ultrasound examination. As well, the two largest pockets of amniotic fluid in a vertical axis from the uterine wall to the fetal trunk were recorded. Amniotic fluid volumes were considered normal when fluid was seen throughout the entire uterine cavity and at least two pockets of >2 cm each were observed.[10]

In each instance the newborn Apgar scores were recorded and the neonatal course in the hospital was documented.

Results

All fetuses had reactive nonstress tests. At each recording at least two pockets of amniotic fluid >2 cm were observed. Each newborn infant had an Apgar score of >7 at 5 minutes and a normal neonatal hospital course.

One hundred thirty-three recorded segments of fetal breathing movements were obtained. A segment terminated either with a bout of apnea or when the intensified dot was displaced from the selected image by fetal stretching or gross body movement or when the transducer position was changed.

In a preliminary study of 95 segments of fetal breathing movements ranging from 11 to 185 breaths (mean = 37) we found that there was a significantly greater proportion of coefficients of variability below the mean (28.3) when the number of breaths in a given segment was <20 than for all other segment lengths. To ensure that the data would not be skewed, a minimum for the number of breaths per segment was set at 20. Seventy-five of the initial 95 segments in this preliminary study contained 20 or more breaths (mean = 43). The mean coefficient of variability was 30.23 in the 75 segments and a regression analysis of the number of breaths versus the coefficient of variability yielded r = 0.09.

Of the 133 tracings obtained in this study 79 had ≥20 breaths and were used for calculation of coefficient

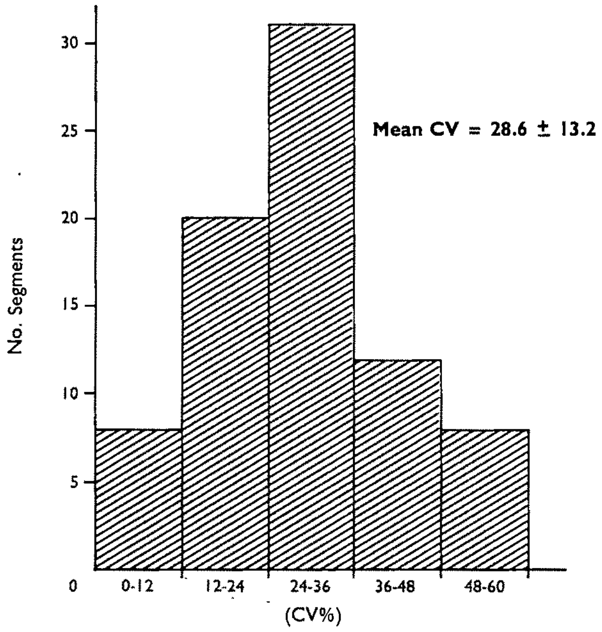

Fig. 2. Distribution of 79 measurements of coefficient of variability *(CV)* over epochs of 12%.

of variability. The mean rate of breathing was 56 ± 11 per minute and the mean coefficient of variability was 28.6% ± 13.2%. The distribution of coefficient of variability values is illustrated in Fig. 2.

Comment

Maloney et al.[11] described the following effects of hypoxia on the pattern of fetal breathing movements: slowing of the rate and alteration of variability to either a regular pattern or an irregular gasping pattern. In fetal heart rate studies, beat-to-beat variability is known to indicate an intact neural pathway and adequate cerebral oxygenation. Loss of this variability reflects a compromised fetus. In our study of normal human fetuses at term combined, direct, on-line graphic recordings of fetal breathing movements and the analysis of breath-to-breath variability confirmed that variability does occur and the normal distribution of the values reflects a biologic phenomenon. It is possible that the diminished variability or irregular gasping pattern described by Maloney et al. occurs for a very brief period preceding prolonged apnea, in which case their detection during random analysis of fetal breathing movements in antenatal patients would be unlikely. Alternatively, they may be preterminal events following a period of prolonged apnea. We have observed slow, irregular gasping patterns in a fetus following intraamniotic hypertonic saline injection and in another fetus at term one half hour prior to an unexplained stillbirth. If these patterns are indeed brief and preterminal then they would rarely be seen in routine antenatal fetal surveillance.

Therefore, while the capability of determining breath-to-breath variability exists, the usefulness of this measurement as a test of fetal health would be contingent on a study of sick fetuses showing a shift in the distribution curve of coefficient of variability values plus a time-related margin of safety which might allow appropriate therapy.

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The distribution of accelerations of the human fetal heart rate at 38 to 40 weeks' gestational age

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In order to further understand the use of antepartum fetal heart rate monitoring we measured the distribution, in time, of two, three, or five fetal heart rate accelerations of ≥ 15 bpm for ≥ 15 seconds and of ≥ 10 bpm for ≥ 6 seconds in 12 healthy pregnant women at 38 to 40 weeks' gestation. The length of time necessary to measure 50% or 95% of intervals containing five accelerations would be substantially reduced by changing to a definition of two or three accelerations. However, an observation interval of at least 80 minutes is required to include the longest time interval of two, three, or five accelerations. These data may suggest new strategies for decreasing time and expense of fetal heart rate testing. (AM J OBSTET GYNECOL 1985;151:283-7.)

Key words: Nonstress test, distribution of fetal heart rate accelerations

The nonstress test (NST) is an important clinical test of fetal health during human pregnancy. The test has been criticized because of low positive predictive values for fetal distress,[1] but most investigators[1-4] have agreed that a reactive NST has a high negative predictive value for fetal health at the time of the test.

It has been demonstrated, during the last third of pregnancy, that the human fetus makes body movements about 10% of the time in episodes that resemble rest-activity patterns observed during sleep in newborn infants.[5] Further, it has been reported that healthy fetuses may not move for periods of 50 to 75 minutes and clinicians will need to account for these periods of inactivity in strategies used for nonstress testing. Finally, it was reported that the incidence of false positive tests was reduced by either increasing the length of continuous observation[4, 6, 7] or by reducing the number of fetal heart rate accelerations with fetal movements required to define fetal reactivity during observation intervals.[2, 8] The purpose of this study was to examine the biologic patterns of fetal heart rate accelerations during normal pregnancies near term and to analyze the distribution of fetal heart rate accelerations in time in order to provide a physiologic basis for the clinical use of antepartum fetal heart rate testing.

Methods

Patients. Informed consent was obtained from 12 healthy pregnant women between 38 and 40 weeks' gestation. Fetal outcome confirmed the good health of fetuses studied and was previously reported.[9] No fetus was delivered within 48 hours of study. The mean birth weight was 3507 gm and there was no 5-minute Apgar score <8. The umbilical cord arterial P_{O_2}, P_{CO_2}, and pH were within the normal range, and there was no umbilical cord arterial pH of <7.20.

One fetus was delivered by repeat cesarean section and one fetus, in a breech presentation, was delivered by cesarean section during labor for failure to progress. None of the 12 fetuses developed fetal distress in labor.

Experimental design. Twelve women were studied continuously for 24 hours from 8:00 AM to 8:00 AM the next day. Studies were conducted in a quiet room exposed to normal daylight and darkness and women rested in bed, either sitting up or in a lateral recumbent position. They were given 800 kcal meals at 8:00 AM, 12 noon, and 5:00 PM. Smoking was not permitted by patients or observers.

Analysis of fetal heart rate and fetal body movements. A mixed fetal and maternal electrocardiogram was obtained from three platinum needle electrodes (Grass Instruments, Quincy, Massachusetts) placed subcutaneously in the anterior abdominal wall of the mother. Fetal and maternal QRS complexes were recognized by an abdominal electrocardiogram processor (Hewlett-Packard Corporation, Palo Alto, California). The time between successive fetal and maternal heart-

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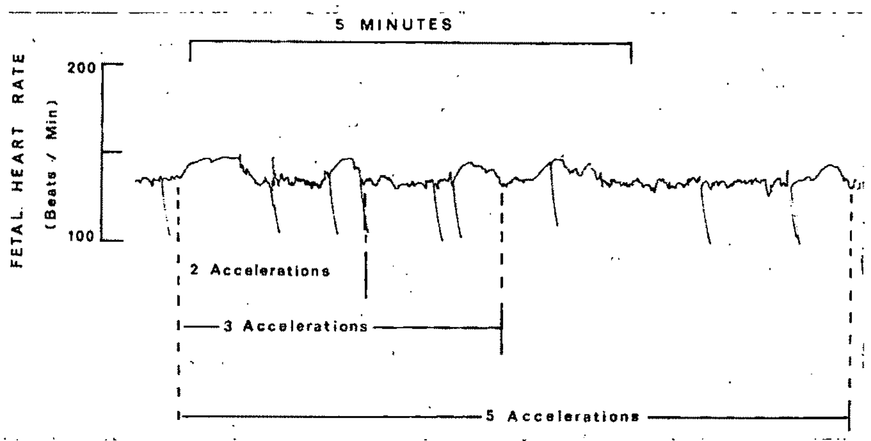

Fig. 1. A chart recording with five fetal heart rate accelerations demonstrates method of analysis used to determine the time necessary to observe two, three, or five fetal heart rate accelerations.

beats, recognized by the processor, were measured on line to within 50 microseconds by a PDP 11 computer (Digital Equipment Corporation, Maynard, Massachusetts) as previously described.[9] The mean FHR and incidence and number of fetal body movements were analyzed on line.

Gross fetal body movements were measured with an ADR real-time scanner (ADR Ultrasound, Tempe, Arizona) as previously described.[5] The transducer operated at a frequency of 3.5 MHz with an average intensity of 0.045 mW/cm^2. Individual gross fetal body movements were identified on a video monitor and coded by an event marker. The transducer was positioned over the maternal abdomen to permit continuous observation of a longitudinal cross section of fetal chest and abdominal wall echoes.

For the purpose of this analysis, accelerations in the fetal heart rate were identified from continuous chart recordings as increases in the fetal heart rate of either ≥15 bpm for ≥15 seconds or ≥10 bpm for ≥6 seconds from the baseline. The baseline was taken as the mean fetal heart rate for 2 minutes before and 2 minutes after accelerations unless a shift occurred in the baseline, in which case the value 2 minutes before was considered the baseline. Only intervals between successive accelerations that were not interrupted by artifact or technical problems were considered for analysis. When successive accelerations were identified, the times between the beginning of one to the end of the next acceleration, the beginning of one to the end of the third, and the beginning of one to the end of the fifth consecutive acceleration were measured (Fig. 1). Data were analyzed after accounting for time during which it was impossible to make analysis because of technical problems or patient interruption (16.2% ± 1.5% of total time). Therefore, the data represent analysis of 241 of the 288 total hours of recording time. The time during which artifact occurred represented times when the patient was in the bathroom or changed position in bed and appeared to be relatively random.

Results are presented as grouped means and standard errors of the mean.

Results

Distribution of intervals of accelerations of 15 bpm for 15 seconds. There were 3,893 intervals during which two successive fetal heart rate accelerations occurred. Fig. 2 is a cumulative histogram of the percentage of intervals during which two accelerations occurred plotted against the length of time of intervals from the beginning of one to the end of the next consecutive acceleration of ≥15 bpm for ≥15 seconds from the baseline. It can be seen that 50% of intervals were <150 seconds (2.5 minutes), 95% of intervals were <480 seconds (8 minutes), but the longest interval containing two consecutive accelerations uninterrupted by artifact was 4200 seconds (70 minutes).

There were 3453 intervals during which three consecutive fetal heart rate accelerations occurred. Fig. 3 is a cumulative histogram of the percentage of intervals during which three accelerations occurred plotted against the length of time of intervals from the beginning of one to the end of the third consecutive acceleration. It can be seen that 50% of intervals were <300 seconds (5 minutes), 95% of intervals were <810 seconds (13.5 minutes), but the longest interval containing three consecutive accelerations uninterrupted by artifact was 4890 seconds (81.5 minutes).

There were 2903 intervals during which five consecutive fetal heart rate accelerations occurred. Fig. 4 is a cumulative histogram of the percentage of intervals during which five accelerations occurred plotted against the length of time of intervals from the beginning of one to the end of the fifth consecutive acceleration. It can be seen that 50% of intervals were <570 seconds (9.5 minutes), 95% of intervals were <1440

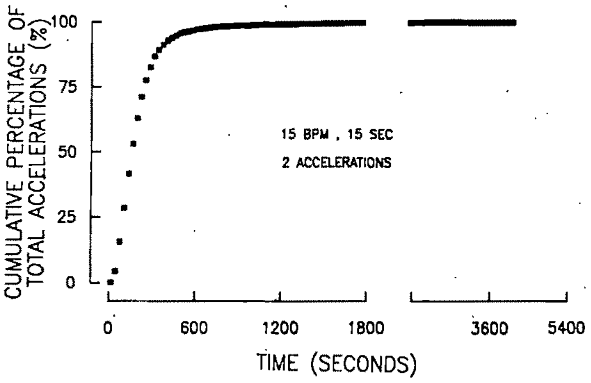

Fig. 2. A cumulative histogram of the percentage of intervals during which two accelerations occurred plotted against the length of time of intervals from the beginning of one to the end of the next consecutive acceleration.

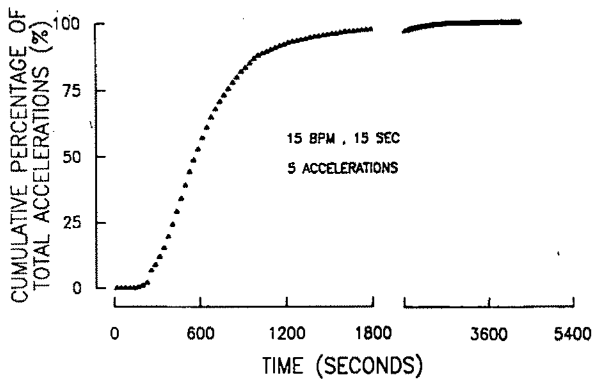

Fig. 4. A cumulative histogram of the percentage of intervals during which five accelerations occurred plotted against the length of time of intervals from the beginning of one to the end of the fifth consecutive acceleration.

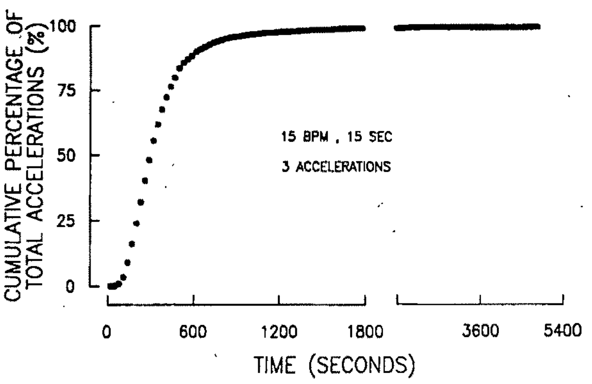

Fig. 3. A cumulative histogram of the percentage of intervals during which three accelerations occurred plotted against the length of time of intervals from the beginning of one to the end of the third consecutive acceleration.

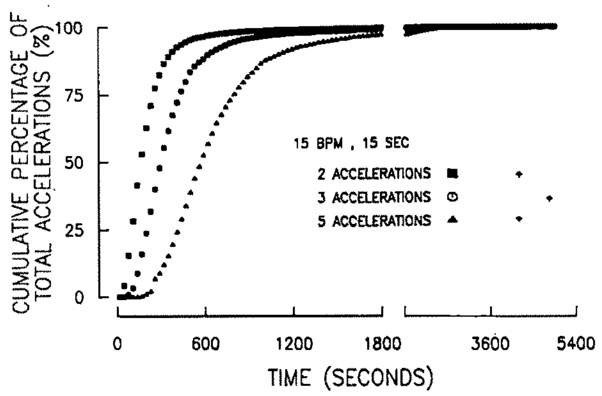

Fig. 5. Cumulative histograms for the length of time necessary to observe two, three, or five fetal heart rate accelerations of ≥15 bpm for ≥15 seconds.

seconds (24 minutes), but the longest interval containing five consecutive accelerations uninterrupted by artifact was 4230 seconds (70.5 minutes).

Fig. 5 is a plot that summarizes Figs. 2, 3, and 4 and demonstrates the difference in distribution of time during which two, three, or five fetal heart rate accelerations of ≥15 bpm for ≥15 seconds occur. It can be seen that the time during which 50% or 95% of intervals occurred was significantly greater for three or five accelerations but that the longest period of time during which two, three, or five accelerations occurred was not significantly different (that is, 70 to 80 minutes).

In order to examine whether a different definition of a fetal heart rate acceleration might influence the distribution of time intervals of accelerations we measured accelerations of ≥10 bpm for ≥6 seconds. It can be seen in Fig. 6 that the time during which two accelerations of ≥10 bpm for ≥6 seconds (n = 5940 intervals) occurred for 50% was 120 seconds (2 minutes), that for 95% of intervals was 390 seconds (6.5 minutes), and that for all intervals was 1950 seconds (32.5 minutes). The time during which three accelerations of ≥10 bpm for ≥6 seconds (n = 5528) occurred for 50% of intervals was 210 seconds (3.5 minutes), that for 95% was 630 seconds (10.5 minutes), and that for all intervals was 2460 seconds (41 minutes). Finally, the time during which five accelerations of >10 bpm for >6 seconds (n = 4828 intervals) occurred for 50% of intervals was 420 seconds (7 minutes), that for 95% was 1050 seconds (17.5 minutes), and that for all intervals was 3060 seconds (51 minutes).

Comment

These data demonstrate the distribution of consecutive fetal heart rate accelerations of ≥15 bpm for ≥15 seconds and of ≥10 bpm for ≥6 seconds measured over 24-hour observation intervals in a group of healthy pregnant women at 38 to 40 weeks' gestational age. For the classical definition of fetal heart rate accelerations ≥15 bpm for ≥15 seconds the longest interval necessary to measure two, three, or five accelerations was 70 to 80 minutes. However, in at least half of time intervals,

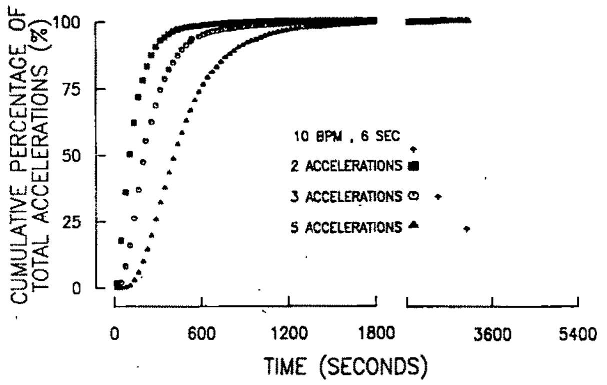

Fig. 6. Cumulative histograms for the distribution of time during which there were two, three, or five fetal heart rate accelerations of ≥10 bpm for ≥6 seconds.

two, three, or five accelerations would be observed within any 10-minute interval. Therefore, if clinicians wish to use the NST as an index of fetal health an improved strategy would be to wait for the presence of either two, three, or five accelerations and then terminate the test. This would save considerable expensive chart paper and professional time in conducting tests. Our data suggest that it would be necessary to observe continuously for 80 minutes in only less than 1% of cases before measuring either two, three, or five accelerations in healthy fetuses. It is important to note that the quality of the fetal heart rate tracing was ideal, and in clinical practice the data would apply only if a high-quality tracing was obtained.

It is interesting that we previously measured the fetal heart rate in a larger group of fetuses in hospitalized patients with high-risk pregnancies and also observed that the longest time necessary to measure five accelerations in any 20-minute interval was 80 minutes.[4]

A physiologic basis for the nonstress test was provided from studies in fetal lambs by Natale et al.[12] who determined that virtually all fetal forelimb activity ceased during isocapnic hypoxemia in mature fetal lambs. Indeed, it appears that virtually all skeletal muscle activity is arrested in the hypoxemic fetus. Human fetuses, during the last 10 weeks of pregnancy, make about 30 gross fetal body movements per hour and spend about 9% to 10% of the time making movements on average.[5] However, episodes of gross fetal body movements occur pseudoperiodically[13] over a broad range of frequencies, and we previously reported from other studies that human fetuses may be at rest for up to 75 minutes during the last 10 weeks of pregnancy.[5] The clinical dilemma is how to separate healthy fetuses during prolonged periods of rest from fetuses not moving because of hypoxemia and/or asphyxia.

Patterns of fetal heart rate accelerations and of gross fetal body movements are essentially the same at term. Timor-Tritsch et al.,[11] using strain gauges placed on the maternal abdominal wall, reported that 99.8% of all fetal movements of >3 seconds were associated with fetal heart rate accelerations. With the use of real-time ultrasound for recognition of fetal movements, it was reported that 85% of all fetal heart rate accelerations occurred with gross fetal body movements and 92% of all gross fetal body movements were associated with fetal heart rate accelerations.[10] It is not yet clear what physiologic mechanism causes the coincidence of fetal heart rate accelerations and gross fetal body movements. However, quantitative observation of fetal movements would appear to be interchangeable with fetal heart rate accelerations as a measurement of reactivity in structurally normal, near term, human fetuses, with mothers not receiving drugs which might reduce or abolish accelerations in the fetal heart rate.

Finally, clinicians have adopted the definition for an acceleration of ≥15 bpm for ≥15 seconds. The current data were collected from highly controlled experiments and demonstrate that a definition of fetal heart rate acceleration of ≥10 bpm for ≥6 seconds would reduce the time necessary to observe fetal heart rate accelerations. However, until improved recording methods appear, the traditional definition should be used to simplify the problem of artifacts interfering with fetal heart rate analysis. Furthermore, considerable clinical data would be required before introducing a new definition of the threshold for an acceleration. It remains to be determined whether similar distributions of fetal heart rate accelerations occur in fetuses early in pregnancy. In particular, we need much more data regarding the fetal heart rate during the second and early third trimesters of pregnancy in humans.

We wish to thank Drs. J. Challis, P. Harding, and B. Richardson for their interest in this work and Mrs. Theresa Clarke for her excellent technical assistance.

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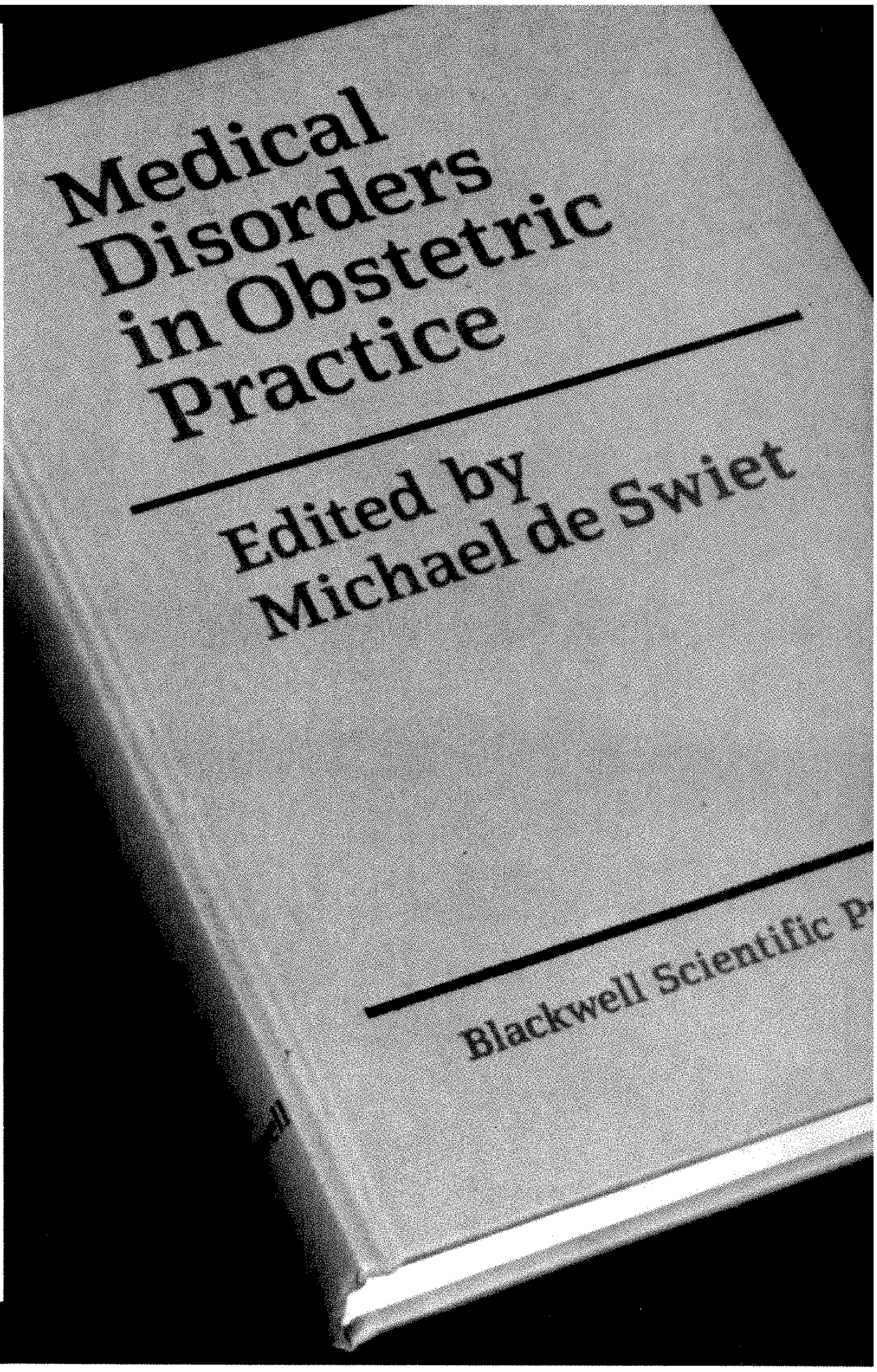
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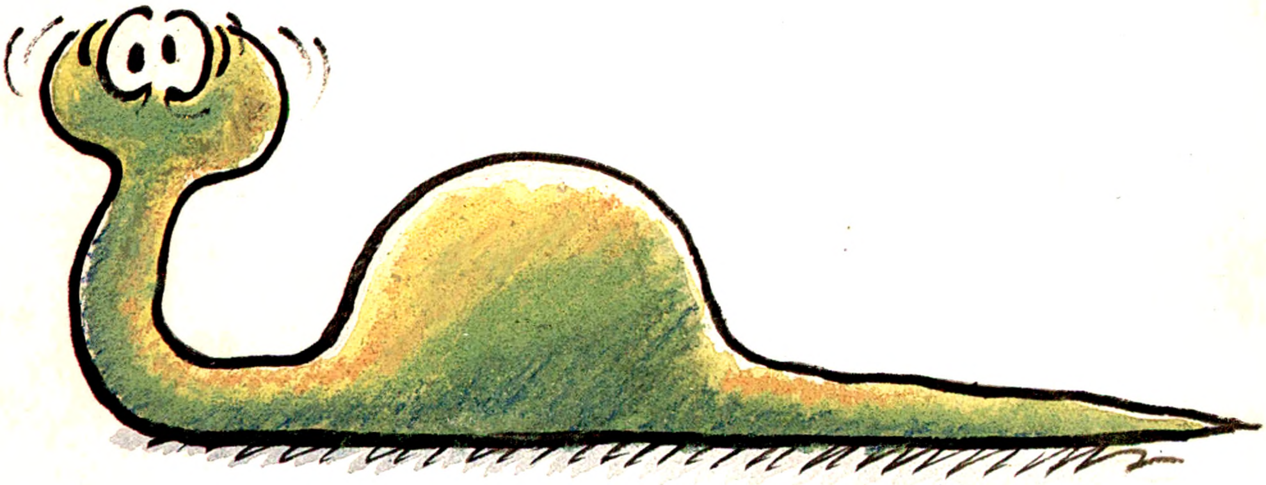

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1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk. C—Use associated with moderate risk.
B—Use associated with high risk. D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

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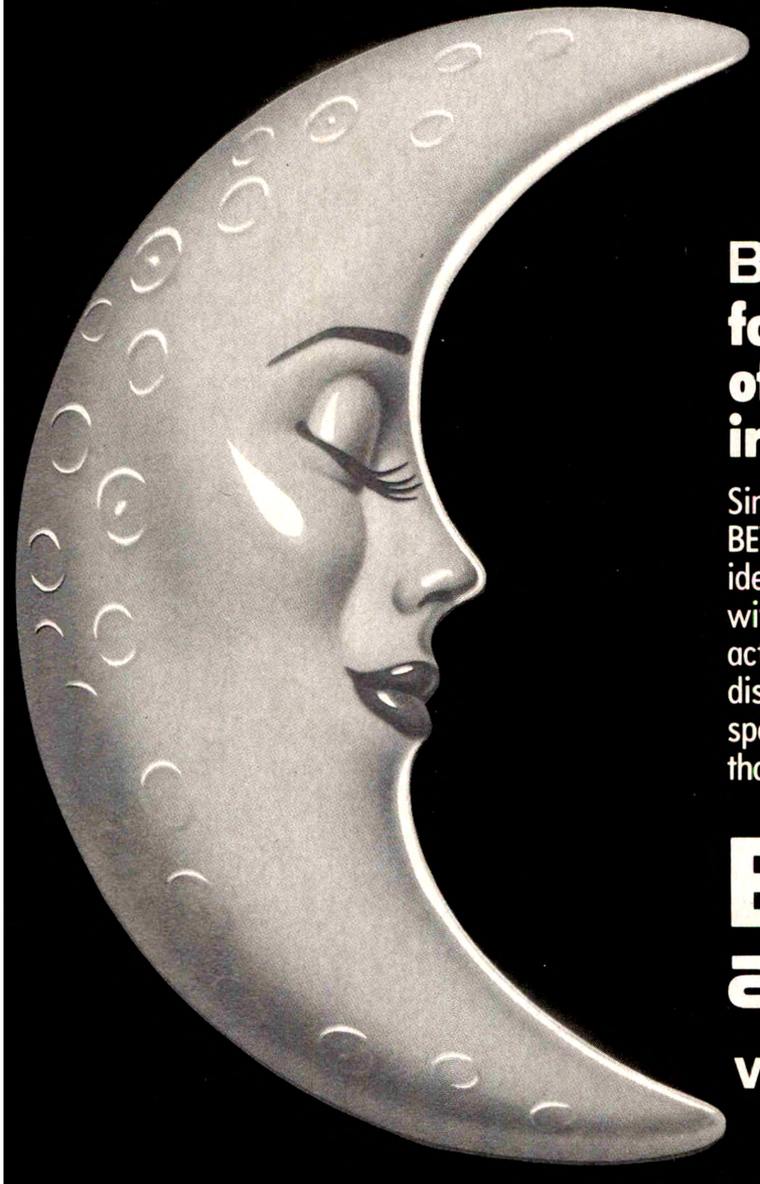

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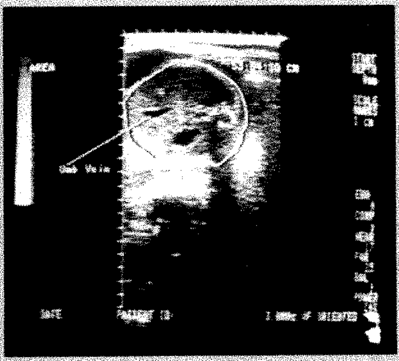

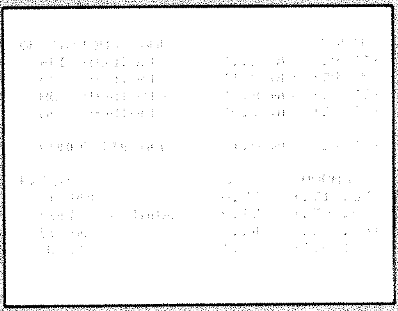

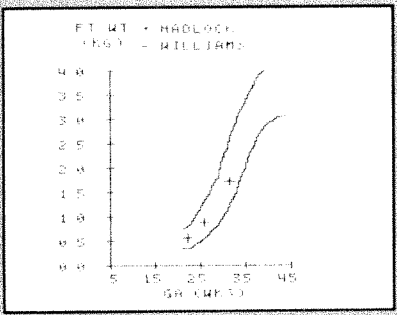

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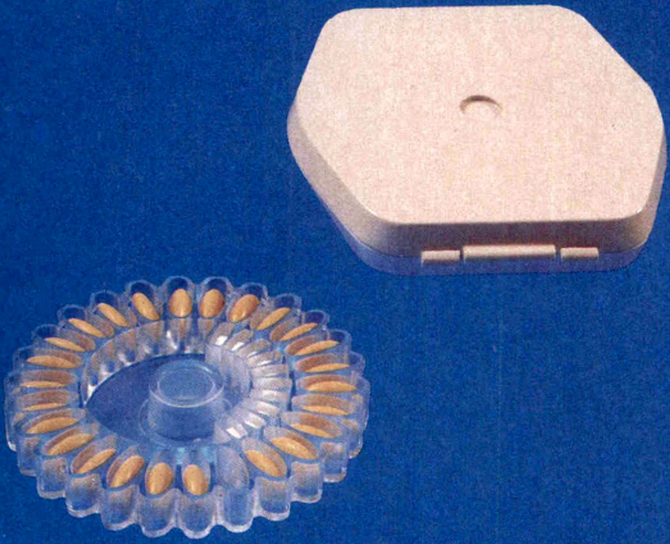

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[1] Dickey RP: Managing Contraceptive Pill Patients, 3rd ed., Durant, Oklahoma, Creative Infomatics, Inc., 1983.

[*] Rates are derived from separate studies from different investigators in several population groups and cannot be compared precisely.

[†] Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.

[‡] Data on file for 22,489 total cycles, Wyeth Laboratories. **See important information on following page.**

IN BRIEF:

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions.

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment, edema, chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum, cholestatic jaundice; migraine; increase in size of uterine leiomyomata, rash (allergic), mental depression; reduced tolerance to carbohydrates; vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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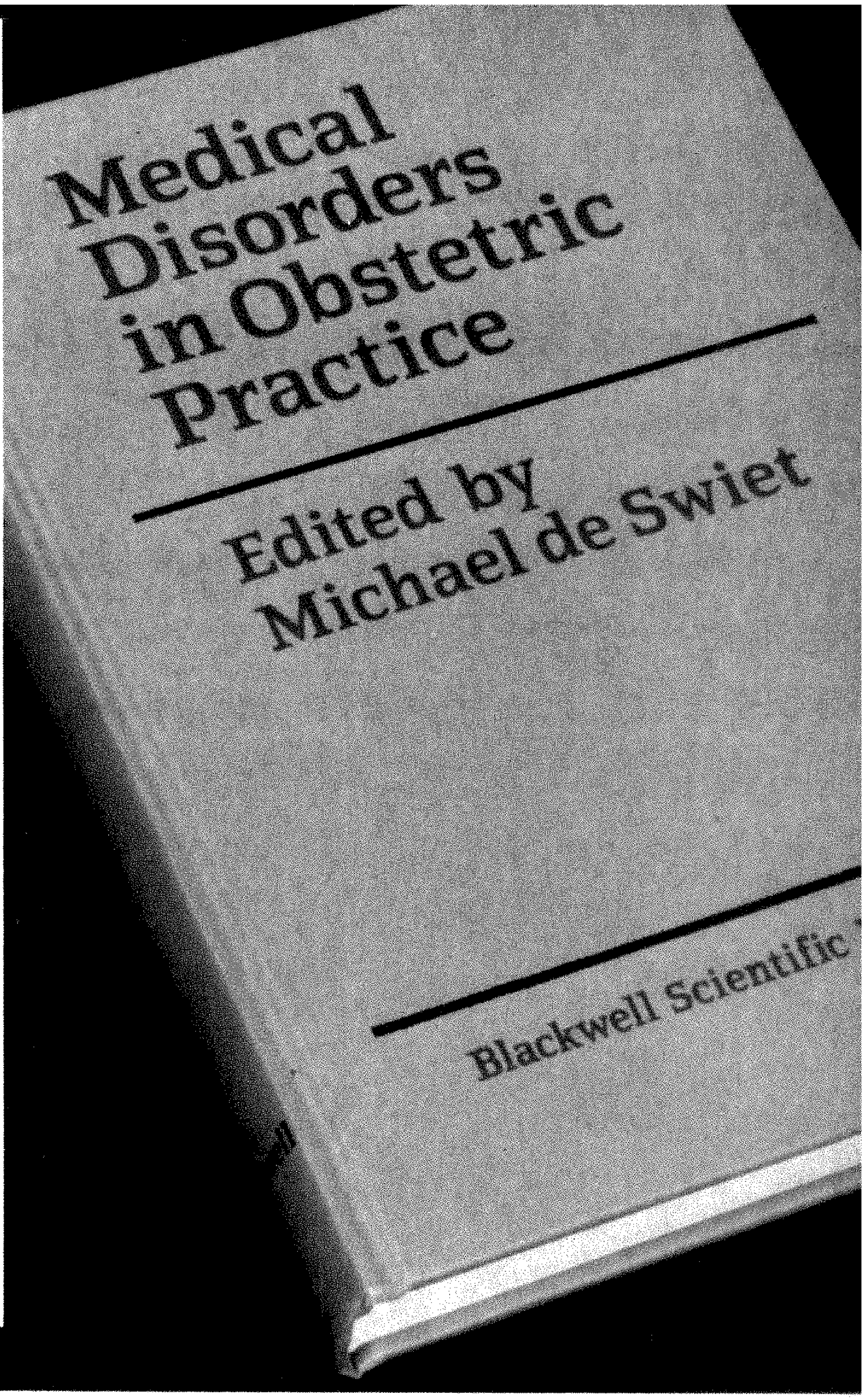
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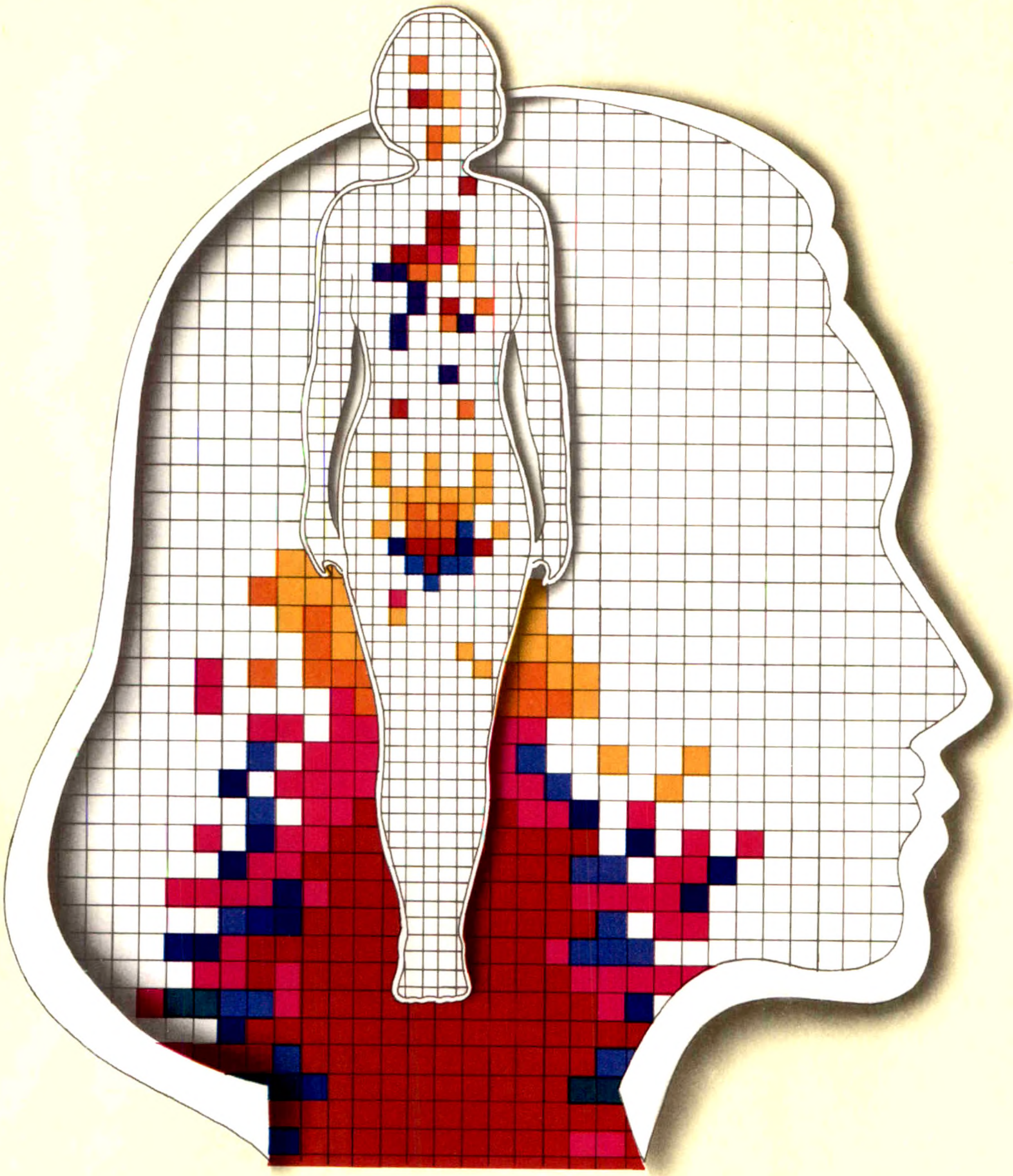

THE ENDOCRINOLOGY OF AGING
Three profiles

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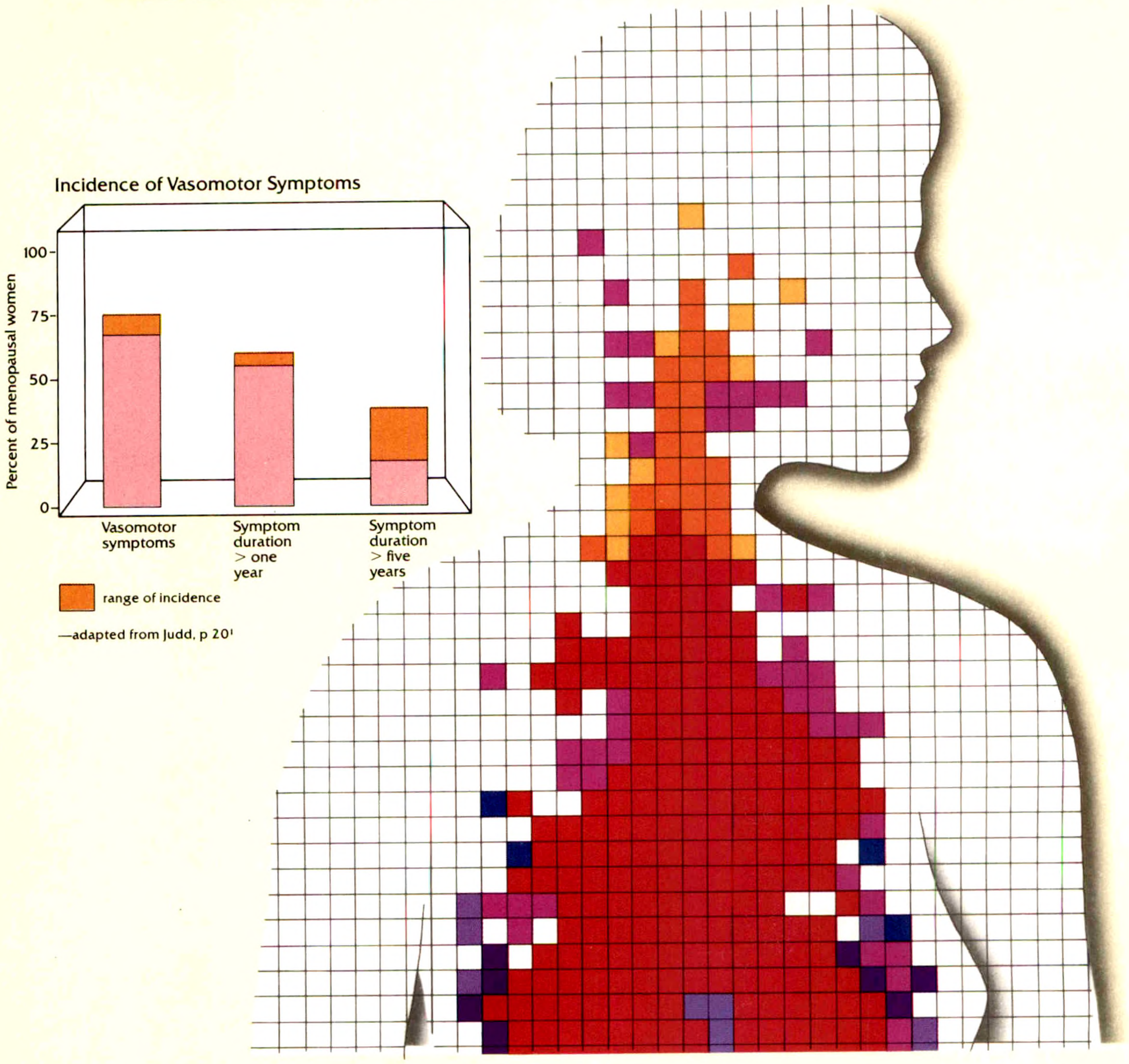

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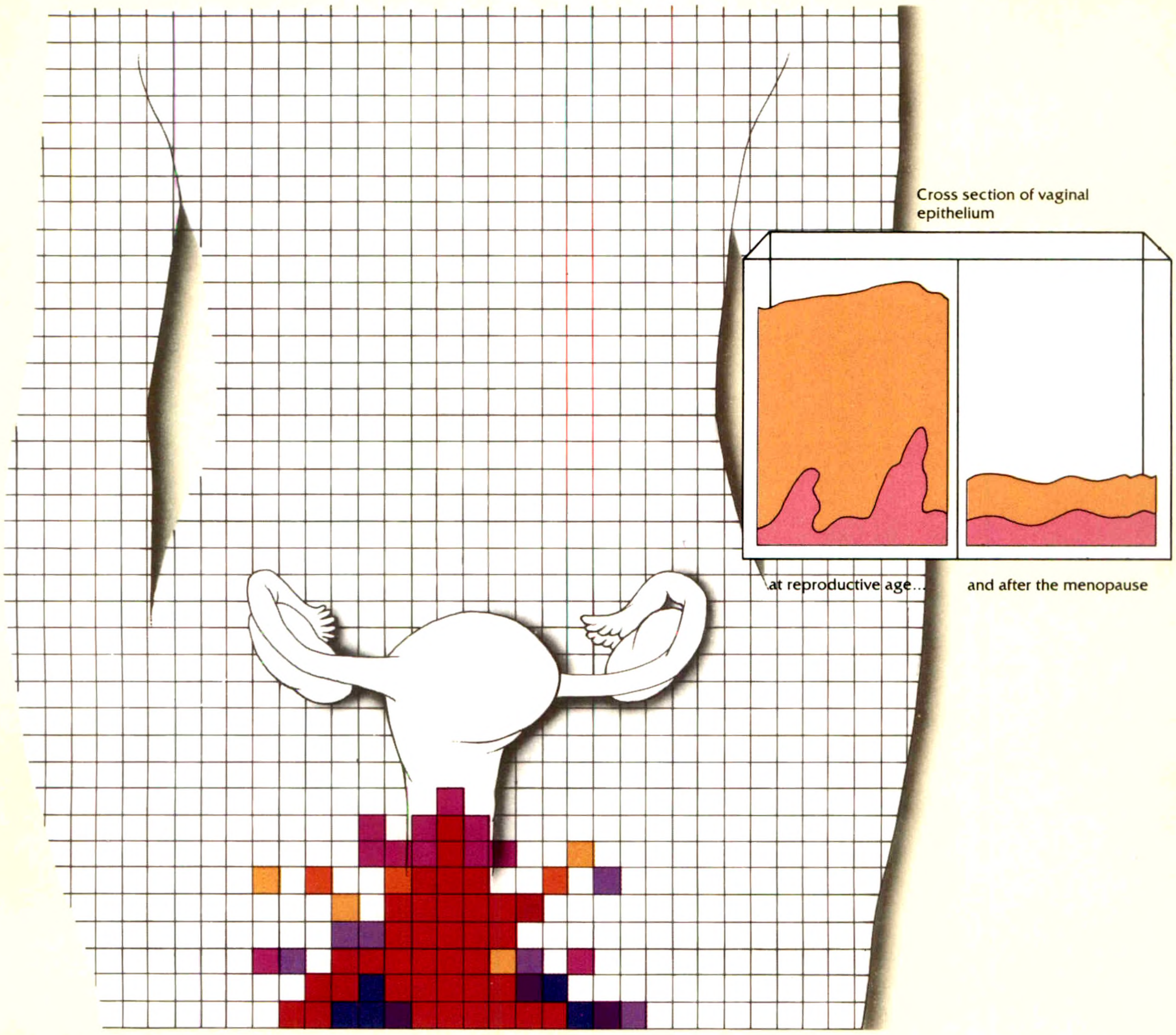

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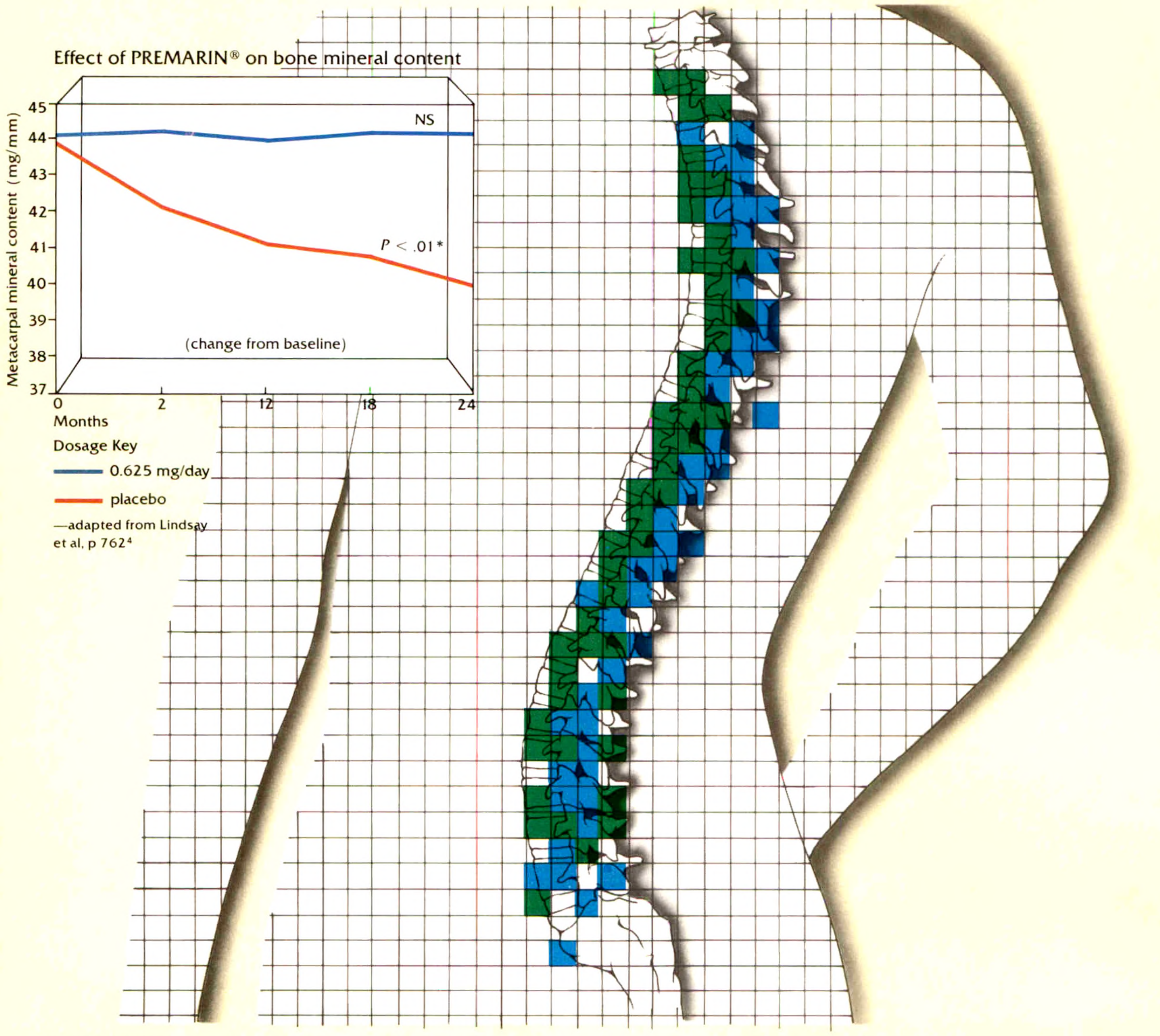
(CONJUGATED ESTROGENS, U.S.P.) Vaginal Cream

0.625 mg/g

Please see last page for brief summary of full prescribing information.

POSTMENOPAUSAL BONE LOSS THAT INCAPACITATES



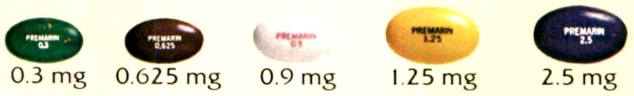
PREMARIN MAY HALT THE DISABLING COURSE OF OSTEOPOROSIS*

Osteoporosis has an enormous epidemiological impact: it affects 10 million American women, and 26% of all women over age 60.[5] The disease begins silently and progresses inexorably for 15 to 20 years, until disabling complications occur.[6]

To minimize osteoporotic damage, the condition must be detected early and treated promptly. For many patients, PREMARIN is optimal therapy for osteoporosis, as part of a comprehensive program that includes exercise, good nutrition, and calcium supplements. In a controlled study of postmenopausal and oophorectomized women, PREMARIN (Conjugated Estrogens Tablets, U.S.P.) doses of 0.625 mg/day prevented loss of metacarpal mineral content (see graph above).

PREMARIN®

(CONJUGATED ESTROGENS TABLETS, U.S.P.)



*Conjugated Estrogens Tablets have been evaluated as probably effective for treating estrogen-deficiency-induced osteoporosis.

Please see last page for brief summary of full prescribing information.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR)*

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

PREMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (Conjugated Estrogens, U.S.P.) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters.

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective: 1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)

2. Atrophic vaginitis
3. Kraurosis vulvae
4. Female hypogonadism
5. Female castration
6. Primary ovarian failure
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma—palliative therapy of advanced disease.
9. Postpartum breast engorgement—Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

INDICATIONS: PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. PREMARIN Vaginal Cream HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Long term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.) At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, although a recent study has raised this possibility. There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

1. *Given cyclically for short-term use only.* For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (*e.g.*, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. *Given cyclically:* Female hypogonadism. Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression)—1.25 mg daily, cyclically.

3. *Given for a few days:* Prevention of postpartum breast engorgement—3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. *Given chronically:* Inoperable progressing prostatic cancer—1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopausal women—10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (*e.g.*, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

Usual dosage range: 2 to 4 g daily, intravaginally or topically, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.). No. 865 – Each *purple* tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866 – Each *yellow* tablet contains 1.25 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864 – Each *white* tablet contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867 – Each *maroon* tablet contains 0.625 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and unit dose package of 100. No. 868—Each *green* tablet contains 0.3 mg in bottles of 100 and 1,000. The appearance of these tablets is a trademark of Ayerst Laboratories.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream—No. 872 – Each gram contains 0.625 mg Conjugated Estrogens, U.S.P. (Also contains cetyl esters wax, cetyl alcohol, white wax, glyceryl monostearate, propylene glycol monostearate, methyl stearate, phenylethyl alcohol, sodium lauryl sulfate, glycerin, and mineral oil.)

Combination package: Each contains Net Wt. 1½ oz. (42.5 g) tube with one calibrated plastic applicator.

Also Available – Refill package: Each contains Net Wt. 1½ oz. (42.5 g) tube. **4340/285**

References: 1. Judd HL: After the menopause. *Transition* 1983;1:19-30. **2.** Erlik Y, Tataryn IV, Meldrum DR, et al: Association of waking episodes with menopausal hot flushes. *JAMA* 1981;245:1741-1744. **3.** Meldrum DR: The pathophysiology of postmenopausal symptoms. *Sem Reprod Endocrinol* 1983;1(February):11-17. **4.** Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 1984;63:759-763. **5.** Katz WA: *Rheumatic Diseases: Diagnosis and Management*. Philadelphia, JB Lippincott Co, 1977, p 672. **6.** Reese WD: A better way to screen for osteoporosis. *Contemp Ob/Gyn* 1983;22(November):116-131.

Ayerst®

AYERST LABORATORIES
New York, N.Y. 10017

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8 ADVANTAGES IN A FIRST-LINE PARENTERAL ANTIBIOTIC

Infection in the hospitalized patient often calls for the immediate intervention of empiric antibiotic therapy. While awaiting culture and sensitivity results,* first-line CEFOBID therapy can provide significant advantages for the patient, the physician, and the hospital:

1. excellent clinical effectiveness†
2. the broadest *in vitro*‡ cephalosporin spectrum—with activity against almost all clinically relevant organisms§, including *Pseudomonas aeruginosa, Enterococcus,* and *Bacteroides fragilis*
3. high peak serum levels
4. therapeutic concentrations in a variety of tissues and body fluids. Body fluid levels and tissue penetration are regarded as essential to therapeutic efficacy, but specific body fluid and tissue levels have not been correlated with specific therapeutic effects.
5. dual pathway of excretion—no adjustment generally required in usual dosage for patients with renal or hepatic impairment
6. excellent safety profile and well tolerated. CEFOBID is contraindicated in patients with known allergy to the cephalosporin class of antibiotics.
7. *b.i.d.* dosing for all indications—usual adult dosage is 1 to 2 g q12h¶
8. substantial administration cost savings because of *b.i.d.* regimen.[1]

CEFOBID® IM/IV q12h

(cefoperazone sodium)

1g and 2g vials

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* Pending results of susceptibility studies, therapy with CEFOBID may be initiated; however, treatment should be adjusted according to bacteriologic findings.

† In infections due to susceptible strains of indicated organisms.

‡ *In vitro* susceptibility does not necessarily imply a correlation with *in vivo* results.

§ Based on PDR, 1984; Manufacturer's Prescribing Information.

¶ In severe infections, or infections caused by less sensitive organisms, the total daily dose and/or frequency *may be* increased. Patients have been successfully treated with a total daily dosage of 6 to 12 grams divided into two, three, or four administrations ranging from 1.5 to 4 grams per dose.

1. Carlin HS, Romankiewicz J: The impact of dosage regimen on intravenous drug cost. Scientific Exhibit, American Society of Hospital Pharmacists, New Orleans, Dec 6-10, 1981.

Please see CEFOBID® brief summary on next page.

Brief Summary

For Intravenous or Intramuscular Use

CEFOBID®

(cefoperazone sodium)

CONTRAINDICATIONS: CEFOBID (cefoperazone sodium) is contraindicated in patients with known allergy to the cephalosporin-class of antibiotics.

WARNINGS: BEFORE THERAPY WITH CEFOBID IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE THE USE OF SUBCUTANEOUS EPINEPHRINE AND OTHER EMERGENCY MEASURES.

PSEUDOMEMBRANOUS COLITIS HAS BEEN REPORTED WITH THE USE OF CEPHALOSPORINS (AND OTHER BROAD-SPECTRUM ANTIBIOTICS); THEREFORE, IT IS IMPORTANT TO CONSIDER ITS DIAGNOSIS IN PATIENTS WHO DEVELOP DIARRHEA IN ASSOCIATION WITH ANTIBIOTIC USE.

PRECAUTIONS: Although transient elevations of the BUN and serum creatinine have been observed, CEFOBID alone does not appear to cause significant nephrotoxicity. However, concomitant administration of aminoglycosides and other cephalosporins has caused nephrotoxicity.

CEFOBID is extensively excreted in bile. The serum half-life of CEFOBID is increased 2–4 fold in patients with hepatic disease and/or biliary obstruction. In general, total daily dosage above 4 g should not be necessary in such patients. If higher dosages are used, serum concentrations should be monitored.

Because renal excretion is not the main route of elimination of CEFOBID (see CLINICAL PHARMACOLOGY), patients with renal failure require no adjustment in dosage when usual doses are administered. When high doses of CEFOBID are used, concentrations of drug in the serum should be monitored periodically. If evidence of accumulation exists, dosage should be decreased accordingly.

The half-life of CEFOBID is reduced slightly during hemodialysis. Thus, dosing should be scheduled to follow a dialysis period. In patients with both hepatic dysfunction and significant renal disease, CEFOBID dosage should not exceed 1–2 g daily without close monitoring of serum concentrations.

As with other antibiotics, vitamin K deficiency has occurred rarely in patients treated with CEFOBID. Those at risk include patients with a poor nutritional status, malabsorption states (e.g., cystic fibrosis), alcoholism, and patients on prolonged hyper-alimentation regimens (administered either intravenously or via a naso-gastric tube). Prothrombin time should be monitored in these patients and exogenous vitamin K administered as indicated.

A disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia has been reported when alcohol (beer, wine) was ingested within 72 hours after CEFOBID administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOBID. A similar reaction has been reported with other cephalosporins.

Prolonged use of CEFOBID may result in the overgrowth of nonsusceptible organisms. Careful observation of the patient is essential.

CEFOBID should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Drug Laboratory Test Interactions: A false-positive reaction for glucose in the urine may occur with Benedict's or Fehling's solution.

Carcinogenesis, Mutagenesis, Impairment of Fertility: The maximum duration of CEFOBID animal toxicity studies is six months. In none of the *in vivo* or *in vitro* genetic toxicology studies did CEFOBID show any mutagenic potential at either the chromosomal or subchromosomal level. CEFOBID produced no impairment of fertility and had no effects on general reproductive performance or fetal development when administered subcutaneously at daily doses up to 500 to 1000 mg/kg prior to and during mating, and to pregnant female rats during gestation. These doses are 10 to 20 times the estimated usual single clinical dose.

Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in mice, rats, and monkeys at doses up to 10 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to CEFOBID. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Only low concentrations of CEFOBID are excreted in human milk. Although CEFOBID passes poorly into breast milk of nursing mothers, caution should be exercised when CEFOBID is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: In clinical studies the following adverse effects were observed and were considered to be related to CEFOBID therapy or uncertain etiology:

Hypersensitivity: As with all cephalosporins, hypersensitivity manifested by skin reactions (1 patient in 45), drug fever (1 in 260), or a change in Coombs' test (1 in 60) has been reported. These reactions are more likely to occur in patients with a history of allergies, particularly to penicillin.

Hematology: As with other beta-lactam antibiotics, reversible neutropenia may occur with prolonged administration. Slight decreases in neutrophil count (1 patient in 50) have been reported. Decreased hemoglobins (1 in 20) or hematocrits (1 in 20) have been reported, which is consistent with published literature on other cephalosporins. Transient eosinophilia has occurred in 1 patient in 10.

Hepatic: Of 1285 patients treated with cefoperazone in clinical trials one patient with a history of liver disease developed significantly elevated liver function enzymes during CEFOBID therapy. Clinical signs and symptoms of nonspecific hepatitis accompanied these increases. After CEFOBID therapy was discontinued, the patient's enzymes returned to pre-treatment levels and the symptomatology resolved. As with other antibiotics that achieve high bile levels, mild transient elevations of liver function enzymes have been observed in 5–10% of the patients receiving CEFOBID therapy. The relevance of these findings, which were not accompanied by overt signs or symptoms of hepatic dysfunction, has not been established.

Gastrointestinal: Diarrhea or loose stools has been reported in 1 in 30 patients. Most of these experiences have been mild or moderate in severity and self-limiting in nature. In all cases, these symptoms responded to symptomatic therapy or ceased when cefoperazone therapy was stopped. Nausea and vomiting have been reported rarely.

Symptoms of pseudomembranous colitis can appear during or for several weeks subsequent to antibiotic therapy (see WARNINGS).

Renal Function Tests: Transient elevations of the BUN (1 in 16) and serum creatinine (1 in 48) have been noted.

Local Reactions: CEFOBID is well tolerated following intramuscular administration. Occasionally, transient pain (1 in 140) may follow administration by this route. When CEFOBID is administered by intravenous infusion some patients may develop phlebitis (1 in 120) at the infusion site.

ROERIG Pfizer

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DRIONIC® THERAPY FOR THE HYPERHIDROTIC

- **MODALITY** — Battery operated device uses no chemicals. Tap water wetted pads are applied to treatment areas. Four to ten treatments will inhibit excess sweat for six week periods.
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WIDE APPLICATION

Hyperhidrosis may predispose to local infections with fungi, bacteria or viruses.[3] Excess sweat presents a social disadvantage, economic burden and a handicap in occupations and sports.[4]

References: 1. Morgan K: Physiotherapy 66:45,1980. 2. Shelley WB et al: J Invest Dermatol 11:275-291,1948. 3. Hurley HJ: *in* Moschella SL et al(eds.): Dermatology Vol 2,Phila.,1975, WB Saunders, p.1166. 4. Shelley WB: Consultations in Dermatology II,Phila.,1974, WB Saunders,p.256. ***on file, General Medical Co.**

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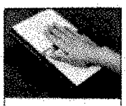

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The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States and Japan, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan).

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It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

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General requirements for preparation of manuscripts

The original and two good-quality copies of the manuscript and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; departmental, divisional, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name. Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

References. Number references consecutively in the order in which they are mentioned in the text. Use the format of the U.S. National Library of Medicine in *Cumulated Index Medicus*. Journal titles should also conform to abbreviations used in *Cumulated Index Medicus*.

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and 2 sets of unmounted prints on glossy paper. For black and white illustrations, submit 3 sets of 5 by 7 inch (8 by 10 inch maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name, title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

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Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

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Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). An average table or figure published in the JOURNAL is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures or used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

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Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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References:
1. Rössner S et al: *Acta Obstet Gynecol Scand* 59:255, 1980. 2. Nash AL et al: *Med J Aust* 2:277, 1979. 3. Larsson-Cohn U et al: *Fertil Steril* 35:172, 1981. 4. Briggs MH: *J Reprod Med* 28:92, 1983. 5. Åhrén T et al: *Contraception* 24:451, 1981. 6. Briggs MH, Briggs M: *Acta Obstet Gynecol Scand* Suppl 105:25, 1982.

***Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.**

See important information on reverse page.

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Indications and Usage—NORDETTE® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See Patient Package Labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See Warnings section #3, 4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NORDETTE® each tablet contains
0.15 mg levonorgestrel and
0.03 mg ethinyl estradiol.

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CLINICAL SECTION

Clinical Opinion

Controversies in Rh prophylaxis

Who needs Rh immune globulin and when should it be given?

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A summary of the current recommendations for administration of Rh immune globulin after several reproductive events is presented. The risks of failure to administer Rh immune globulin after delivery or abortion, amniocentesis, and massive transplacental hemorrhage are described. (AM J OBSTET GYNECOL 1985;151:289-94.)

Key words: Rh immune globulin, abortion, amniocentesis, pregnancy complications

An Rh-negative woman delivered of an Rh-positive, ABO-compatible baby has a 16% likelihood of becoming Rh immunized as a result of her pregnancy. A total of 1½% to 2% of mothers will be overtly Rh immunized by the time they are delivered. Another 7% will have demonstrable Rh immunization within 6 months after delivery. The remaining 7% will first show that they were Rh immunized by mounting a secondary Rh immune response in their next Rh-positive pregnancy. To use the term coined by Nevanlinna,[1] the latter were "sensibilized" by the first pregnancy, primary Rh immunization being too weak to be detected.

ABO incompatibility confers partial protection against Rh immunization. The Rh-negative woman who is delivered of an Rh-positive, ABO-incompatible baby is at 1½% to 2% risk of becoming manifestly Rh immunized by 6 months after delivery.

Standard Rh prophylaxis

The development and licensure in 1968 of Rh immune globulin, an effective means of preventing Rh immunization and therefore of preventing erythroblastosis and hydrops fetalis, were milestones in preventive medicine. Rh immune globulin should always be successful in preventing Rh immunization if it is given before Rh immunization has begun and if it is given in sufficient dose. The standard intramuscular dose in North America is 300 μg. In other parts of the world intramuscular Rh immune globulin doses of 100 to 125 μg have proved to be almost equally effective. In Canada, Ireland, and parts of Germany intravenous Rh immune globulin preparations are given in doses of the order of 100 to 120 μg.

Because all clinical trials upon which the licensure of Rh immune globulin was based involved administration of Rh immune globulin 72 hours after delivery, the original recommendation was that one prophylactic dose should be given intramuscularly within 72 hours after birth of an Rh-positive infant irrespective of its ABO status.

Since we have observed rare but undoubted examples of Rh immunization becoming manifest within 72 hours after delivery, our present policy is to give Rh immune globulin as soon after delivery as the Rh status of the baby has been determined and the mother is confirmed to be at risk of Rh immunization.

If for some reason the Rh status of the baby is not known by 72 hours after delivery, we administer Rh immune globulin to the mother despite the one third chance that her baby is Rh-negative and that she is not at risk. We prefer to treat a woman unnecessarily rather than withhold Rh immune globulin from a mother who is at risk of Rh immunization. Conversely, if through an oversight a woman is left unprotected, we will give her Rh immune globulin up to 28 days after delivery as soon as we have discovered the oversight. Samson

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Table I. Residual Rh prophylaxis problems

1. Failure to administer Rh immune globulin after delivery or abortion
2. Failure to give Rh immune globulin after amniocentesis
3. Failure to prevent Rh immunization after massive fetal transplacental hemorrhage
4. Rh immunization during pregnancy—antenatal Rh prophylaxis
5. Rh immunization in infancy—the "grandmother theory"
6. The mother with weak Rh antibodies
7. The problem of the D^u mother

and Mollison[2] have shown that volunteers given Rh-positive red blood cells are partially protected when Rh immune globulin is given up to 13 days after exposure. The patient and her physician must understand, however, that the longer after delivery Rh prophylaxis is delayed, the less likely it is that she will be protected.

Since abortion carries a significant risk of Rh immunization (2% if the abortion is spontaneous and 4% to 5% if the abortion is therapeutic), we administer Rh immune globulin to every Rh-negative unimmunized woman who aborts. If a 50 μg Rh immune globulin minidose is available it will be protective if abortion occurs in the first trimester. Unless fetal transplacental hemorrhage screening is carried out after abortion, we recommend that a standard dose of Rh immune globulin be given if therapeutic abortion is carried out in the second trimester (50 μg of Rh immune globulin will protect only against a transplacental hemorrhage of up to 2 ml of fetal red cells).

Standard Rh prophylaxis has been about 85% to 90% effective in eradicating Rh immunization. Table I lists remaining problems which will be discussed in order.

Failure to administer Rh immune globulin after delivery or abortion. Failure to protect the Rh-negative mother after delivery of an Rh-positive baby may be due to her failure to seek prenatal care, failure of her physician to send blood for grouping and antibody screening, or failure of the hospital obstetric unit to send maternal and cord blood for testing after delivery.

Medical and nursing personnel must know the Rh and alloimmunization status of all of their pregnant patients. The Rh-negative woman should have blood rescreened at 20, 28, and 32 to 34 weeks' gestation. At delivery maternal and cord blood must be tested. If the baby is Rh-positive and the mother Rh-negative and not Rh immunized, Rh immune globulin must be given. A weakly positive cord red blood cell direct antiglobulin test in the presence of ABO incompatibility is never a reason for withholding Rh immune globulin. The infant has ABO erythroblastosis. ABO incompatibility does not prevent Rh immunization completely. Rh immune globulin must be given.

The Rh-negative woman who becomes Rh immunized after abortion does so after a small red blood cell exposure. She is a good responder. She frequently has a very severely affected fetus in her next Rh-positive pregnancy. No woman should have a therapeutic abortion until her Rh status is determined. If she is Rh-negative and unimmunized, Rh immune globulin must be given at the time of abortion.

Compliance after spontaneous abortion is more difficult. The woman who aborts spontaneously may do so before her first prenatal visit. She may not be hospitalized or her stay may be very brief. The Rh-negative woman should be aware of the risks of Rh immunization after spontaneous abortion. Personnel caring for the woman who aborts spontaneously must determine her Rh status and give her Rh immune globulin if she is Rh-negative and unimmunized.

The Rh-negative woman who has an antepartum hemorrhage without loss of the conceptus is at substantial risk of transplacental hemorrhage and Rh immunization, although there are no statistics available to determine exactly what the increased risk is. We recommend that an Rh-negative woman who has an antepartum hemorrhage should be given a full prophylactic dose of Rh immune globulin and that the dose be repeated every 12 weeks until delivery.

Failure to give Rh immune globulin after amniocentesis. If the placenta is traversed at amniocentesis there is a significant hazard of fetal transplacental hemorrhage (10.6% in our experience before the ultrasound era). Although ultrasound reduces the risk of placental trauma at amniocentesis it does not remove it completely, nor does a negative Kleihauer acid elution or any other fetal cell screen rule out transplacental hemorrhage altogether. Every Rh-negative unimmunized woman undergoing amniocentesis (for genetic purposes in early pregnancy, for pulmonary maturity determination in later pregnancy) must be given a full prophylactic dose of Rh immune globulin which again should be repeated every 12 weeks until delivery. Although there was originally a fear that administration of Rh immune globulin after amniocentesis might harm the conceptus, there is abundant evidence that a dose of 300 μg is harmless.

Failure to prevent Rh immunization after massive transplacental hemorrhage. There is now evidence that one standard dose of Rh immune globulin (300 μg) will prevent Rh immunization following exposure to up to 25 ml of fetal Rh-positive blood (12 ml of fetal red blood cells). Following exposure to as much as one unit (450 ml) of Rh-positive blood, 300 μg of Rh immune globulin reduces the incidence of Rh immunization from the expected 70% to about 35%.

Fetal transplacental hemorrhage exceeding 30 ml occurs rarely, being found in only 0.23% of 9000 pregnant women studied in Winnipeg. The great majority of these transplacental hemorrhages occur during delivery. If fetal transplacental hemorrhage screening were never carried out and 300 μg of Rh immune globulin were given routinely, Rh immunization due to undiagnosed massive transplacental hemorrhage would occur in only one Rh-negative woman in 1250 (0.08%) at risk.

It is apparent that failure to diagnose and treat massive transplacental hemorrhage is a rare cause of residual Rh immunization. Because accurate measurement of fetal transplacental hemorrhage is difficult for a routine hospital laboratory and because Rh immunization due to massive fetal transplacental hemorrhage is a rare event, an argument can be made for not carrying out fetal screening for transplacental hemorrhage. However, we do screen for fetal transplacental hemorrhage and it is recommended by obstetric and blood banking societies.

If the diagnosis of massive fetal transplacental hemorrhage is made after delivery, our policy is to give 300 μg of Rh immune globulin intramuscularly for hemorrhage of less than 25 ml of Rh-positive fetal blood, 600 μg for 25 to 49 ml, 900 μg for 50 to 74 ml, etc. We give the contents of four vials (1200 μg) every 12 hours until the calculated dose has been given.

In the very rare event when a transplacental hemorrhage exceeding 75 to 100 ml (we have seen up to 450 ml) has been diagnosed before delivery, fetal compromise due to anemia as well as the risk of Rh immunization must be considered. Fetal condition should be assessed by ultrasound. If the mother is past 33 weeks' gestation prompt delivery should be undertaken with neonatal resources available to treat the baby if he is in shock or severely anemic. If the baby is Rh-positive, the mother should be given Rh immune globulin in the dose already outlined.

If an Rh-negative patient is deliberately or accidentally transfused with Rh-positive blood, attempts at Rh prophylaxis may cause significant anemia. If more than 900 ml of Rh-positive blood (450 ml of packed red blood cells) has been given, Rh prevention should not be attempted unless the patient is a girl or a woman in her childbearing years. In this situation we carry out a one and a half blood volume exchange transfusion with ABO-compatible Rh-negative blood less than 4 days old, which removes in excess of 75% of the circulating donor Rh-positive blood. Rh immune globulin is then administered in a dose calculated on the basis of 25% to 30% of the original volume transfused. Differential agglutination 48 to 72 hours after the final dose should show no residual Rh-positive red blood cells and antibody screening should indicate the presence of passive Rh antibody.

Rh immunization during pregnancy and antenatal Rh prophylaxis. Rh immunization occurring during pregnancy and therefore not prevented by postdelivery Rh prophylaxis is the present cause of the greatest amount of residual Rh immunization. It causes 20 times as much residual Rh immunization as does undiagnosed massive fetal transplacental hemorrhage. The Manitoba study[3] showed that 62 of 3533 women (1.8%) not Rh immunized at the beginning of their pregnancies were Rh immunized at delivery or within 3 days after delivery. Similar findings were reported from Hamilton, Ontario, and Sweden[4] and in primigravid women from Yorkshire, England.[5]

Such Rh immunization carries with it serious consequences. In 24 subsequent Rh-positive pregnancies, 11 babies (46%) required phototherapy and/or exchange transfusions; five of the 11 (21% of the total) were so severely affected that they required either early delivery (two) or fetal transfusions and early delivery (three).

An antenatal prophylaxis clinical trial, 300 μg of Rh immune globulin given at 28 and again at 34 weeks' gestation, carried out in Winnipeg from December, 1968, to August, 1976,[3] was highly successful, reducing the incidence of Rh immunization during pregnancy from 1.8% to 0.1%.

A service program in which 300 μg of Rh immune globulin is given at 28 weeks' gestation has been just about as successful.[6] Of 9609 Rh-negative women given Rh immune globulin at 28 weeks' gestation and subsequently delivered of Rh-positive babies, without antenatal prophylaxis we would have expected 159 to be Rh immunized by the end of their pregnancies, manifesting their immunization by delivery, or within 6 months after delivery, or in some not until a secondary immune response was mounted in a subsequent pregnancy. Nineteen instances of Rh immunization were found, and in 10 of the 19 there was an explanation for the immunization other than failure of antenatal prophylaxis (Rh immunization before 28 weeks' gestation in a subsequent pregnancy in two; "sensibilization" due to failure to carry out antenatal, postnatal, or postabortion prophylaxis in or after prior pregnancies in eight). The true antenatal prophylaxis protection rate is therefore 150 of 159 (94.3%). In three of the nine "true" failures the intervals between antenatal and postnatal Rh prophylaxis were 13 weeks, 5 days, 14 weeks, 3 days, and 15 weeks, respectively.

For the above reasons our policy, which we recommend strongly to others, is to give 300 μg of Rh immune globulin at 28 weeks' gestation to the unimmunized Rh-negative pregnant woman whose husband is Rh-positive or Rh unknown. Because of the hazards of "augmentation," which we believe to be very real if circulating anti-D drops to very low levels,[7] we administer a second dose of Rh immune globulin if delivery has not occurred within 12 weeks, 3 days after the first prenatal injection. In this instance, if delivery occurs within 3 weeks after the second antenatal injection and if there is no Kleihauer evidence of a fetal transplacental hemorrhage in excess of 0.1 ml of fetal red blood cells, we do not administer a postdelivery dose of Rh immune globulin.

Four arguments have been advanced against antenatal Rh prophylaxis[8, 9]: (1) The incidence of Rh immunization during pregnancy is very low; (2) antenatal Rh prophylaxis is not cost effective; (3) hyperimmunization and plasmapheresis may be hazardous to Rh plasma donors; (4) the IgG in Rh immune globulin given to the mother, to which the fetus is exposed, may be hazardous.

Our experience and the experience of the Hamilton and Swedish investigators are that the prevalence of Rh immunization during pregnancy is not low (1.6% to 1.9%). If postpartum and postabortion Rh prophylaxis compliance is high, in the absence of an antenatal Rh prophylaxis program, Rh immunization during pregnancy is by far the leading cause of residual Rh immunization.

The consequences of Rh immunization during pregnancy, as already noted, may be very serious. Five subsequent affected fetuses of the 62 women in our original study were at risk of death and three required intrauterine transfusions. In the 5-year period ending March 15, 1984, 52 women were referred to the Winnipeg Rh Laboratory for intrauterine transfusion. Twelve of the 44 (27.3%) in whom a cause could be found were Rh immunized during a previous pregnancy. Rh immunization during a previous pregnancy shares pride of place with immunization in the pre-prevention era as the most common cause of Rh immunization leading to intrauterine transfusion.

On the basis of an Rh immune globulin cost of $35.00 per dose, postdelivery Rh prophylaxis is one of the most cost effective of all prevention programs, the drug cost to prevent one case of Rh immunization developing after delivery being about $300. Because one and a half times as many doses of Rh immune globulin are required (one third of treated mothers will have Rh-negative babies) to prevent one seventh as many cases of Rh immunization, it will cost 11 times as much to prevent one case of Rh immunization developing during pregnancy (about $3300). This cost is very worthwhile when one considers that 25% of subsequent affected babies will be at risk of fetal death and will require multiple antibody titers, fetal assessments, and amniocenteses. One half of the 25% (13% of Rh-affected Manitoba fetuses in the 5-year period ending October 31, 1983) will require intrauterine transfusion in order to survive.

The $3300 cost compares favorably with the cost of screening for massive transplacental hemorrhage at delivery. Based upon our experience that at least 1250 screening tests are required to prevent one case of Rh immunization due to massive transplacental hemorrhage, it is quite apparent that the cost of screening to prevent one case of Rh immunization due to massive transplacental hemorrhage is at least as great as, indeed, depending upon the screening test used, probably much greater than, the cost of Rh immune globulin to prevent one case of Rh immunization during pregnancy. Since all critics of antepartum Rh prophylaxis are in favor of routine screening for massive transplacental hemorrhage, cost effectiveness is not an argument against but is an argument in favor of antenatal prophylaxis.

The arguments against antenatal prophylaxis because of theoretical risks to donors from hyperimmunization with Rh-positive red blood cells[8] are specious. In 16 years of hyperimmunizing Rh-immunized sterile women (well over 150 in all), not one has shown any deleterious effect. If carefully grouped red blood cells incompatible for D but compatible for C, E, Kell, and Fy^a are administered, atypical antibody formation is rarely encountered and if found, is inconsequential. Some of our plasma donors have undergone plasmapheresis more than 40 times yearly for 16 years. None has shown any untoward side effects. In some there is a modest lowering of total protein and IgG levels but these levels very rarely fall below 60 and 5.5 gm/L, respectively. Plasmapheresis in Winnipeg, once weekly in 30 donors, produces in excess of 1000 L of high-titer Rh plasma annually. This is an amount which vastly exceeds the needs of our country (10% of the population of the United States). We calculate that 300 donors with the same quality of anti-D plasma, undergoing plasmapheresis 40 times a year (8000 liters of plasma), would meet the entire needs of the United States for Rh immune globulin for antenatal as well as postnatal and postabortion prophylaxis. When one considers that more than 5 million plasmaphereses a year are carried out in the United States, the 12,000 plasmaphereses required to meet American needs for universal antenatal, postnatal, and postabortion Rh prophylaxis is a small number indeed.

The argument that exposure of the fetus to passive Rh immune globulin (containing all kinds of IgG) might affect its subsequent immunologic status adversely[9] is also without foundation. All fetuses are exposed to much larger amounts of foreign IgG produced by their mothers. Maternal IgG has a protective effect and does not have a subsequent deleterious effect. There is no reason to expect the small amount of IgG which crosses the placenta after the mother is given Rh immune globulin to have a damaging effect on the fetal and neonatal immune system. We have not studied the immunologic status of our 16,000 babies delivered after their mothers were given antenatal Rh immune globulin and do not intend to do so but we have had no untoward effects reported to us.

Rh immunization in infancy—the "grandmother" theory. It has been suggested that Rh immunization which appears during a first pregnancy may be a secondary immune response antibody following Rh immunization during infancy because of a reverse transplacental hemorrhage of maternal Rh-positive red blood cells at the time of delivery. Sixty percent of mothers of Rh-negative babies are Rh-positive. Studies of capillary blood samples drawn from babies at 1 or 2 days of age indicate about a 2% incidence of maternal-fetal transplacental hemorrhage.[10] The amounts are very small, being the equivalent of a fetal-maternal transplacental hemorrhage of about 0.005 ml (one twentieth the amount associated with a 3% incidence of maternal Rh immunization). The reports of an astounding incidence of neonatal Rh immunization ranging from 5% to 22% have not been substantiated.[11] In our own studies we found no evidence of Rh immunization in 94 Rh-negative babies born of Rh-positive mothers who were screened by enzyme and AutoAnalyzer methods up to 6 to 12 months of age.

If Rh immunization during infancy were the cause of Rh immunization appearing during a first pregnancy, we would expect to see it in 60% of the original 5% to 22% reported (3.0% to 13.2%) rather than the 1.8% observed. We would not expect the 1.8% to have been reduced to 0.1% (nine of 9609), since Rh immune globulin prevents only the primary Rh immune response and not the secondary Rh immune response. Rh immunization due to reverse maternal transplacental hemorrhage, if it occurs at all, is a very rare event. Rh immune globulin should not be given to Rh-negative babies born of Rh-positive mothers.

The mother with weak Rh antibodies—attempts at suppression. On occasion an Rh-negative woman will be discovered to have a weak Rh antibody. Rh antibodies detected only by an AutoAnalyzer technique in most instances do not represent true Rh immunization. They have been found in nontransfused Rh-negative men[12] and, in our experience, showed no increase in strength in 87% of Rh-negative women delivered of Rh-positive babies. Therefore, Rh-negative women with Rh antibodies detectable only by an AutoAnalyzer technique should be given Rh immune globulin during pregnancy and after delivery if they are delivered of Rh-positive babies.

The woman with an Rh antibody demonstrable by a manual enzyme method as well as by an AutoAnalyzer method is in an entirely different situation. In Winnipeg 21 of 36 women given 300 μg of Rh immune globulin at 6-week intervals during pregnancy because of the presence of such a weak antibody had a progressive increase in anti-D levels.[13] When four women without progression who were not challenged by a subsequent Rh-positive pregnancy were excluded, 66% (21 of 32) of women progressed to full-blown Rh immunization and had babies with erythroblastosis fetalis.

The incidence of progression of Rh immunization in 131 pregnant Rh-negative women with similar, very weak antibodies not treated with Rh immune globulin (determined from a retrospective study of 3372 consecutive Rh-immunized women) was also 66% (86 of 131). Thus Rh immune globulin does not suppress Rh immunization when very weak Rh immunization is present and is not recommended. However, if screening tests are equivocal and if the specificity of the antibody is in question, Rh immune globulin should be given, with the clear understanding that it may not be protective.

The problem of the D^u mother. The D^u mother who is delivered of an Rh-positive baby is not at significant risk of Rh immunization. Indeed the majority of so-called D^u mothers are genetically Rh-positive, the weakened expression of D being due to the presence of C or E on the opposite chromosome. They are at no risk of Rh immunization. The true genetic D^u missing a part of the D-antigen mosaic is at very slight risk of Rh immunization. In 40 years of experience, encompassing the screening of more than 750,000 pregnancies, the Rh laboratory has encountered, at most, four or five instances in which a D^u mother produced anti-D. In none of the cases was the infant severely affected. There is one well-documented report of hydrops fetalis in an Rh-immunized D^u mother.[14] The true D^u mother clears Rh-positive red blood cells more slowly than the Rh-negative mother. Rh immune globulin may not be protective. The usual recommendation is that the D^u mother not be given Rh immune globulin. If the uncommon genetic D^u mother can be differentiated from the much more common environmental D^u (Rh-positive) mother, we recommend that she be given Rh immune globulin after delivery, although the risk of Rh immunization is exceedingly small.

Current Rh prophylaxis recommendations. The following are currently recommended.

1. Every Rh-negative unimmunized woman must be given one prophylactic dose of Rh immune globulin, irrespective of the ABO status of the infant, as soon after delivery as the infant has been determined to be Rh-positive. Administration should not be delayed beyond 72 hours. However, if it is inadvertently omitted it should be given up to 4 weeks after delivery.

2. Every Rh-negative nonimmunized woman who has a spontaneous or induced abortion should be given Rh immune globulin unless the father is known to be Rh-negative. A dose of 50 μg, if available, will be protective in the first trimester.

3. Every Rh-negative nonimmunized woman under-

going amniocentesis should be given one prophylactic dose of Rh immune globulin (300 μg) unless the father is known to be Rh-negative. The dose should be repeated in 12 weeks if she has not been delivered of her infant or in 6 weeks if she has had further amniocenteses.

4. Every Rh-negative nonimmunized woman should receive one prophylactic dose of Rh immune globulin (300 μg) at 28 weeks' gestation unless the father is known to be Rh-negative. The dose should be repeated in 12 to 12½ weeks if she has not been delivered of her infant.

5. Every Rh-negative nonimmunized woman who has a fetal transplacental hemorrhage exceeding 25 ml of fetal blood should be given at least 300 μg of Rh immune globulin for every 25 ml of fetal blood in her circulation, unless the father or baby is known to be Rh-negative. Four vials (1200 μg) should be given intramuscularly every 12 hours until the total dose is given.

6. Every Rh-negative woman who has an Rh antibody detectable only by an AutoAnalyzer technique should be treated as if she had no detectable Rh antibody. The Rh-negative woman who has an Rh antibody detectable by a manual enzyme method and/or weakly by an indirect antiglobulin (Coombs) technique will not be protected against progressive Rh immunization by administration of Rh immune globulin. However, she should be treated if the specificity of the antibody is in doubt.

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Maternal death associated with sickle cell trait

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A case report is presented of a patient with sickle cell trait who died an abrupt intrapartum death precipitated by probable peripartal cardiomyopathy and apparently aggravated by intravascular sickling. Notable autopsy findings included massive sicklemia in all organ systems. (AM J OBSTET GYNECOL 1985;151:295-7.)

Key words: Sickle cell trait, maternal death, peripartal cardiomyopathy

Sickle cell trait has been reported only twice as a complication of pregnancy leading to maternal death: a sudden death in an eclamptic patient[1] and death due to massive postpartal pulmonary embolus.[2] It is generally accepted, however, that an increased incidence of urinary tract infection and pyelonephritis does accompany sickle cell trait in pregnancy.

The following is the report of a case managed on the Louisiana State University Obstetrics Service of the Earl K. Long Memorial Hospital in Baton Rouge, Louisiana:

Case report

A 17-year-old black primigravid adolescent was followed up from 18 weeks' gestation with mild anemia and confirmed sickle cell trait. At 36 weeks' gestation she presented with a blood pressure of 130/70 mm Hg (previously 118/70 mm Hg) and proteinuria (3+). The patient was treated with bed rest.

At 37 weeks she presented with ruptured membranes for 4 hours. She had no bleeding or contractions but admitted to a cough for 2 weeks and "feeling hot" for 1 week. The blood pressure was 148/90 mm Hg; respirations, 24 per minute; irregular pulse at 90 bpm, and oral temperature, 99.4° F. Breathing was unlabored, although she was intermittently coughing up pinkish sputum. Auscultation revealed rales in both lower lung fields and an irregular heart rate. Uterine size was consistent with 36 to 37 weeks, and the fetal heart rate was 160 bpm. The cervix was thick and closed, with leaking membranes. There was slight pretibial edema and reflexes were 1+/4+ without clonus.

Admitting diagnoses were: (1) Intrauterine pregnancy of about 36 to 37 weeks, (2) premature rupture of the membranes, (3) preeclampsia, (4) cardiac arrhythmia, and (5) pneumonia or pulmonary edema. Pitocin induction of labor was initiated and magnesium sulfate was administered. Laboratory results were: hemoglobin, 10.2 gm/100 ml; hematocrit, 31.6%, white blood cell count, 11,000/mm^3, with normal differential count, electrolytes, and clotting studies. Electrocardiogram showed sinus tachycardia with frequent ventricular premature beats. Chest x-ray film revealed right pleural effusion, perihilar edema, a patchy infiltrate in the right upper lobe, B lines of Kerley, and full left cardiac silhouette. Arterial blood gas values were pH, 7.44; Pa_{CO_2}, 29.4 torr; Pa_{O_2}, 98.9 torr; and bicarbonate, 19.2. The medical consultant considered pulmonary edema secondary to preeclampsia versus atypical pneumonia, along with possible mitral and/or aortic valvular disease. Furosemide, hydralazine, erythromycin, and lidocaine were administered.

Six hours after admission, the cervix was 2 to 3 cm dilated and 90% effaced. The patient's respirations increased to 40 per minute and the blood pressure was 160/112 mm Hg. Oxygen was administered. Ninety minutes later, marked fetal heart rate decelerations occurred with persistent fetal bradycardia of 40 bpm. There was no response to maternal oxygenation or position changes. Cesarean section was performed with the patient under general endotracheal anesthesia. A female infant was delivered with Apgar scores of 1, 6, 7, and 9 at 1, 5, 10, and 15 minutes, respectively. After delivery the mother experienced bradycardia, ventricular fibrillation, and asystole. Cardiopulmonary resuscitation was performed, but the patient never generated a palpable pulse or blood pressure and was pronounced dead 60 minutes later.

Pertinent autopsy findings included an enlarged heart, with the left atrium significantly dilated and the valves within normal limits. Microscopic examination showed interstitial myocardial edema, focal fragmentation of fibers, minimal lymphocytic infiltration, and some eosinophilia in the sarcoplasm; the capillaries contained sickle cells. The lungs (see Fig. 1) contained pleural effusions and foamy, viscid material in the airways without obstruction. Microscopic examination showed

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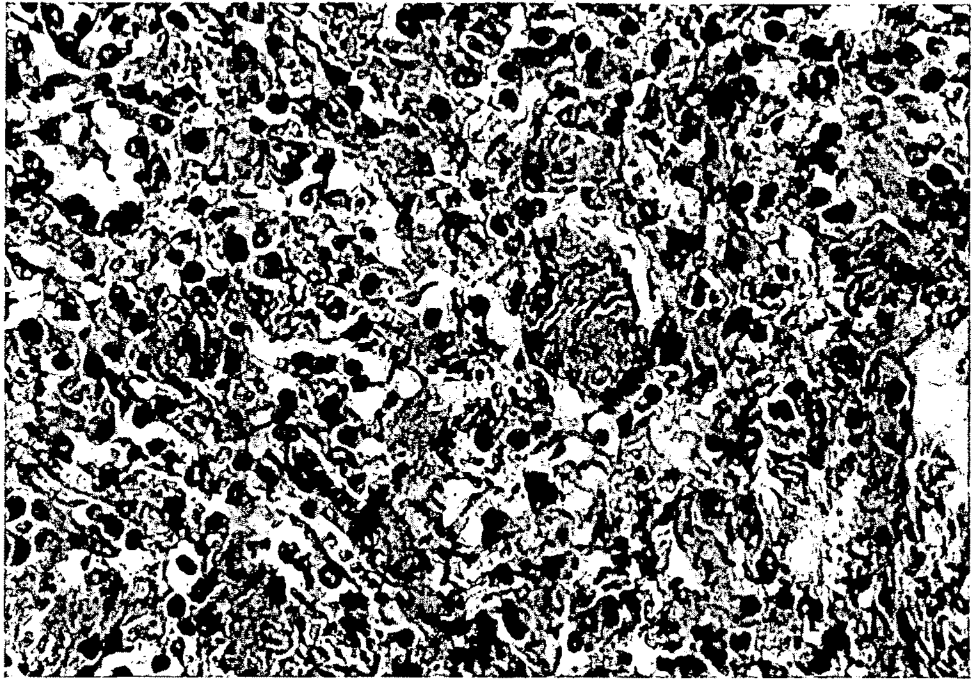

Fig. 1. Pulmonary alveoli showing extensive vacuolated, eosinophilic, cellular intraalveolar edema fluid. There are agglutinated sickle cells within the veins and arteries. (Hematoxylin and eosin stain, oil immersion.)

vacuolated, eosinophilic, cellular, intraalveolar edema fluid with concomitant agglutination of sickle cells in the veins and arteries. The kidneys were of normal size with a pale cortex and hyperemic pyramids. Microscopic examination showed diffuse membranous thickening in most tufts and sickle cells in the glomeruli. There was a 2 mm cortical nodule in the left adrenal; the medulla of each gland was partially autolyzed. Agglutinated sickle cells were present throughout the vasculature. The liver weighed 2100 gm and had a normal gross appearance. On microscopic examination, sickled and normal erythrocytes were seen within the sinusoids. The brain was diffusely edematous. The pathologic diagnoses were: (1) congestive heart failure with acute pulmonary edema, (2) probable cardiomyopathy, (3) cerebral edema, (4) shock kidneys, (5) probable early pneumonia, (6) small cortical nodule of the adrenal, (7) massive intravascular sickling of the internal organs, and (8) membranous glomerulonephritis.

Comment

Approximately 8% of black Americans have hemoglobin AS, or sickle cell trait. Survival in patients with AS hemoglobin is similar to that of patients with AA hemoglobin.[3] Studies of exercise tolerance and athletic performance in persons with sickle cell trait have not shown any compromise in individuals with hemoglobin AS. A recent report, however, presented a case of a cross-country runner with sickle cell trait who had central nervous system dysfunction, disseminated intravascular hemolysis, rhabdomyolysis, renal insufficiency, and deep venous thrombosis presumably brought on by sickling under conditions of extreme stress, lactacidosis, hypoxia, and dehydration.[4] Hematuria, hyposthenuria, and renal papillary necrosis, which are common in patients with sickle cell trait, are thought to be related to these same causes.

Besides the present case, two maternal deaths associated with sickle cell trait are reported in the literature.[1, 2] In these two cases widespread intravascular sickling was demonstrated at autopsy. The reports intimated that the sickling phenomenon played a large part in the outcomes of these two patients. This assumption has been debated with the claim that the presence of sickle cell trait in these women was merely coincidental, since one in 12 American black women may be expected to have hemoglobin AS.

Our patient fits the varied clinical and autopsy picture of peripartal cardiomyopathy. The fact that autopsy revealed massive intravascular sickling does not per se prove that the patient died as a result of the sickling phenomenon; the argument may be made that the intravascular sickling was only an agonal event. It is our opinion, however, that the patient initially became ill because of the cardiomyopathy; when she became sufficiently hypoxic and acidotic, she developed massive intravascular sickling, leading to the final outcome.

Although sickle cell trait is common during pregnancy, severe problems are rarely attributed to it alone. These reported cases, however, indicate that individuals with sickle cell trait may indeed experience sickling under conditions of severe metabolic stress. The obstetrician must therefore consider that a sickle cell trait patient who is severely compromised by preexisting disease, especially hypoxia, acidosis, or other forms of metabolic stress, may possibly undergo sickling which hampers or completely foils all efforts to remedy the original illness. Whether this assumption is true and exactly which patients are at greatest risk await further observation and investigation.

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Trial of labor in the patient with a prior cesarean birth

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The increasing incidence of cesarean birth has become quite controversial. The practice of deliverying virtually all women with a prior cesarean section by repeat cesarean section is open to debate. In a large institutional practice, with rapid availability of all support services, selective attempts to deliver women vaginally, after a prior cesarean section, is appealing. A prospective 1-year study on all patients with a prior cesarean delivery commenced July 1, 1982. There were 1209 patients, of whom 751 (62%) underwent a trial of labor and 614 (82%) achieved vaginal delivery. There was no maternal mortality or perinatal mortality attributed to the trial of labor process. Oxytocin usage occurred in 38% of this population. The incidence of uterine rupture and scar dehiscence was similar in the various study subgroups. The overall group of women undergoing a trial of labor had significantly fewer postpartum complications and shorter hospital stays. (AM J OBSTET GYNECOL 1985;151:297-304.)

Key words: Prior cesarean section, trial labor

The escalating rate of cesarean birth in recent years has focused attention on many factors. The relative safety of cesarean delivery has prompted a more liberal approach to its use with rates of 20% to 30% being common in certain institutions.[1] Attention of the medical professions was focused on this topic following the National Institutes of Health Consensus Development Task Force on cesarean birth. The results of its deliberation were published in October of 1981.[2]

An area of suggested evaluation concerned the pregnancy management and delivery method following a prior cesarean birth. "The obstetrical norm of 'once a c-section, always a c-section' held for more than nine women in ten in 1980, ..."[3] Retrospective reviews have demonstrated the relative safety of selectively permitting vaginal delivery after a cesarean birth.[4-6] A recent comprehensive review paper surveyed 30 years of the English literature and presented the cumulative outcome from approximately 5300 vaginal births following a trial of labor.[7] The authors state: "Properly conducted vaginal delivery after cesarean section is relatively safe, with a 0.7% incidence of uterine rupture, 0.9% perinatal mortality, and no maternal deaths due to uterine rupture."

Recently published studies have demonstrated the safety of attempting vaginal delivery following cesarean birth in contemporary practice but relatively small numbers limit their impact.[8, 9] Of interest is the high success of permitting a trial of labor in view of prior diagnoses such as cephalopelvic disproportion and "failure to progress."[10]

In a large delivery service, many factors prompt the evaluation of attempting vaginal delivery after a prior section. First, the facility with a large volume will often have the necessary support services to attempt a

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Table I. Morbidity measures and hospital stay comparisons for study groups

	Trial of labor (N = 751)				*No trial of labor (N = 458)*					
	Successful, vaginal delivery (n = 614)		*Failure, cesarean section (n = 137)*		*Vaginal delivery (n = 40)*		*Elective cesarean section (n = 157)*		*Other—cesarean section (n = 261)*	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Dehiscence	11	1.8	5	3.6	2	5	4	2.5	7	2.7
Febrile morbidity	14	2.3	37	27	2	5	23	25	49	19
Hospital stay (days)	2.3		4.3		2.3		4.2		4.3	

Elective repeat cesarean section was performed in 13% of the total population with prior cesarean section.

trial of labor in what has been defined to be a safe fashion.[4,5,11] Second, the net result of achieving vaginal delivery in such patients would be a significant cost-saving effect. Third, an increasing number of patients are requesting an attempt at vaginal delivery and thus patient satisfaction might be achieved. Fourth and finally, when several patients who have had a prior cesarean section are admitted in labor, a more rational approach could be undertaken with selective priority being assigned to the patient in greatest need of repeat cesarean section.

In order to evaluate the management of patients with a prior cesarean section, a retrospective review of patients delivered at the Los Angeles County/University of Southern California Medical Center was performed for the year 1980. Following this evaluation it was concluded that the procedure of a trial of labor was not associated with unacceptable risk or major catastrophic complications for the mother or fetus.[12] Thus, a more liberal approach toward offering a trial of labor to patients with a prior cesarean section commenced in July, 1982. The purpose of this report is to provide observations and outcome measures related to the first year of this ongoing prospective evaluation.

Material and methods

All patients in this evaluation were delivered at the Los Angeles County/University of Southern California Medical Center at Women's Hospital, which is the teaching institution of the University of Southern California. During the 1-year period of July, 1982, through June, 1983, there were 16,200 births in this facility, which primarily serves patients of a low socioeconomic class. Trial of labor following a prior cesarean section was activity endorsed with the exception of the following: (1) patients having had a known previous classical uterine incision; (2) those having had more than one previous cesarean section; (3) those with multiple gestation; and (4) patients having a malpresentation other than breech. The previous medical records and historical information were often difficult to obtain and patients often presented for care late in pregnancy. Often the previous delivery had been in Mexico or Latin America and the patients' recollection and language may have compromised the accuracy of these data. However, eligible patients were instructed regarding the known risks and benefits of a trial of labor and given the option to undergo a trial of labor or have a repeat cesarean delivery. A limitation within our institution was that only a single elective cesarean section could be scheduled on four weekdays. Thus most patients electing cesarean section were admitted after onset of early labor or spontaneous rupture of the membranes. Intrapartum care was provided by the house staff in consultation with staff faculty as necessary. Patients received routine nursing care, and continuous electronic monitoring was used to provide fetal heart rate and uterine activity information. Immediate postpartum intrauterine examination was routinely done in an attempt to find uterine dehiscence in those patients who achieved vaginal delivery. Dehiscence was defined as a palpable and/or visualized uterine defect. It was further subgrouped into dehiscences that required no intervention and those that did require intervention, which were termed uterine rupture.

Initial data were collected from the patient's chart, logbooks, and house staff while the patient remained in the hospital and a code sheet was completed for data entry. Statistical evaluation was by χ^2 analysis.

Results

During the study year, a total of 1776 (11%) cesarean sections were performed in the 16,200 women delivered. Overall, there were 1202 primary (7%) and 555 repeat (4%) cesarean sections. This includes patients who underwent a repeat cesarean section for the following reasons: failed trial of labor, 137; elective repeat cesarean section, 157; and repeat cesarean section in labor without a trial, 261. The total number of patients having had one or more prior cesarean births was 1244

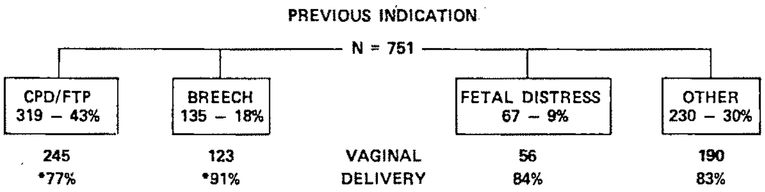


Fig. 1. The reasons for previous cesarean section in patients undergoing a trial of labor and succeeding in vaginal delivery are given. * = $p < 0.05$.

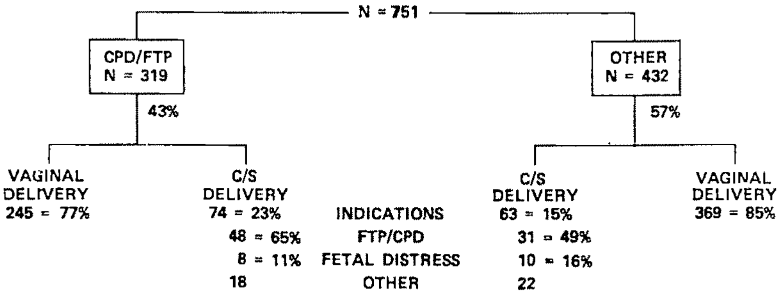


Fig. 2. The outcome of trial of labor in patients with a previous cesarean section for cephalopelvic disproportion is depicted.

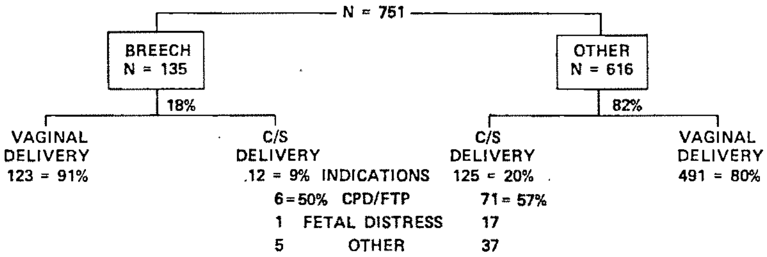
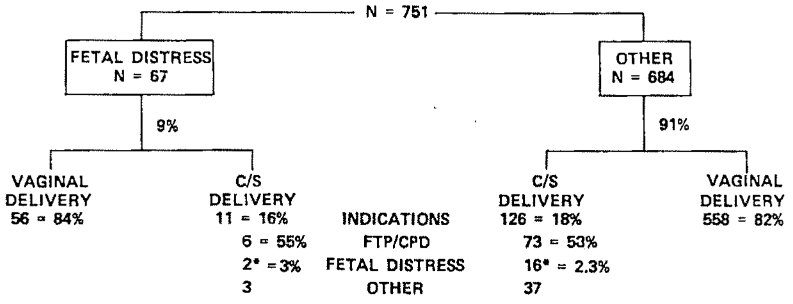


Fig. 3. The outcome of trial of labor in patients with a previous cesarean section for breech presentation is depicted.

or 7.7% of the total births. Of the 1244 patients with a prior cesarean section, 35 patients were excluded for the following reasons: multiple gestation, nine, and unknown intent as to a patient's desire for a trial of labor, 26. Multiple gestations were excluded because on several occasions the second twin was delivered by cesarean section following the vaginal delivery of the first infant. The numbers of prior cesarean sections for these 1209 patients were one, 923; two, 226; three, 52; and four or more, eight. Of the 1209 study patients (Table I), 751 (62%) elected to undergo a trial of labor. Sixty-two patients who met criteria for a trial of labor elected to undergo a repeat cesarean section. In the trial of labor group, these patients had the following number of prior cesarean sections: one, 726; two, 23; three, two. Overall, 592 patients (79%) who underwent a trial of labor had an unknown type of uterine scar. Six hundred fourteen (82%) of these patients were delivered vaginally. One hundred thirty-seven women had failure of a trial of labor and underwent cesarean section. The most common indication for cesarean section was cephalopelvic disproportion/failure to progress occurring in 79 patients (58%). A successful trial of labor by the number of prior cesarean sections was as follows: one, 82%; two, 70%; three, 100%. During a trial of labor, 86 (12%) patients received an epidural anesthetic. In the remaining 458 patients who did not undertake a trial of labor, 40 (9%) were delivered vaginally prior to our being able to perform a cesarean section.

Trial of labor

Previous indications. The success rates of trial of labor as it relates to previous indications for cesarean section are shown in Fig. 1. The group with the significantly lower success rate, compared to all others, was in the group of women who had had a prior cesarean delivery for failure to progress or cephalopelvic disproportion (FTP/CPD) (Fig. 2). This was the most common previous indication, yet 77% of the women with this previous diagnosis achieved vaginal birth. The significantly highest success rate, 91%, occurred in the 135 patients with a previous indication of breech presentation (Fig. 3). The trial of labor results in patients having previ-



*N.S. 3% vs. 2.3%

Fig. 4. The outcome of trial of labor in patients with a previous cesarean section for fetal distress is depicted.

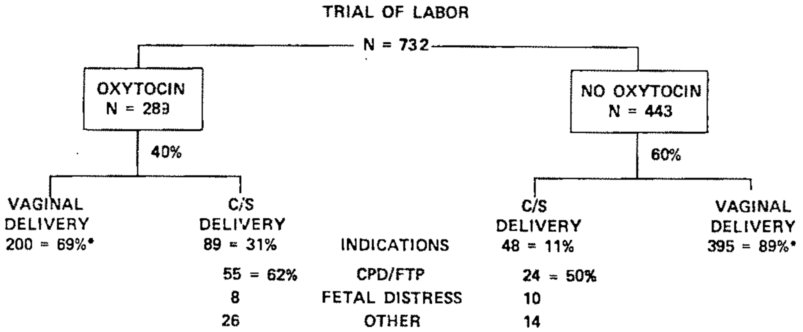


Fig. 5. The outcome of patients that received oxytocin during a trial of labor is contrasted with the group that did not. * = $p < 0.05$.

ously had a cesarean birth for fetal distress are presented in Fig. 4. It should be noted that the incidence or recurrence of fetal distress in this group was not different from that in those patients with other indications for previous cesarean section.

Oxytocin administration. Oxytocin was administered to 289 (38%) patients electing to undertake a trial of labor. Thirty-two patients underwent indicated induction of labor and 257 received oxytocin augmentation in order to achieve adequate uterine activity. Vaginal delivery was achieved in 23 cases with induction (72%) compared to 177 cases with augmentation (69%, NS). The success rate of achieving vaginal delivery in all patients receiving oxytocin was significantly less than that in those who did not receive oxytocin (Fig. 5).

Prior vaginal delivery. The outcome of patients undergoing a trial of labor with a prior vaginal birth is depicted in Table II. The success rate of women having had a prior vaginal birth was significantly better ($p < 0.05$) than that in those who had not had a previous vaginal delivery (88% and 78%, respectively). When the previous vaginal birth occurred subsequent to a cesarean delivery the success rate was 94% ($p < 0.01$).

Current pregnancy outcome

Breech presentation in current pregnancy. There were a total of 72 patients with breech presentations in this pregnancy (Fig. 6). Of these, 13 (18%) underwent a trial of labor. Vaginal delivery was achieved in six (46%). Overall, patients with breech presentations had the lowest incidence of a trial of labor (13 of 72, 18%), successful trial of labor (six of 13, 46%), and vaginal delivery (seven of 72, 9.7%).

Fetal distress in current pregnancy. There were 25 patients in whom cesarean delivery occurred for the diagnosis of fetal distress in the current pregnancy (Fig. 7). Eighteen of these deliveries for fetal distress occurred in patients undergoing a trial of labor and seven in the group in whom cesarean delivery was planned. Of the seven patients in whom the diagnosis of fetal distress was coded, a further evaluation was attempted to define the urgency of cesarean section. The criterion chosen was the preoperative skin preparation which was termed "emergent." Two of the seven patients had such an "emergent" skin preparation. Thus two emergency cesarean sections occurred in the 458 patients with planned cesarean section, an incidence of 0.4%.

Of the 18 cases of fetal distress occurring during a trial of labor, seven cases were termed emergency on the basis of the skin preparation. Thus seven of the 751 (0.9%) undergoing a trial of labor required an emergency delivery. The occurrence of fetal distress in the trial of labor group was not significantly different from that in the group of women in whom cesarean

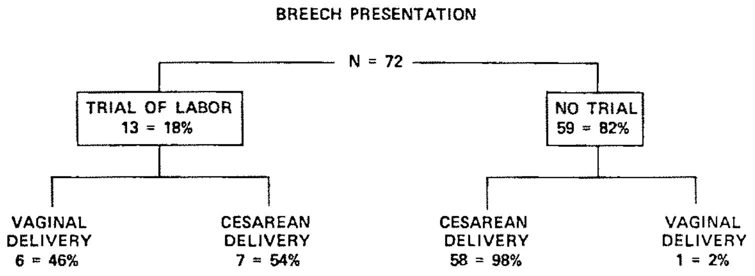


Fig. 6. The outcome of patients with a breech presentation in the current pregnancy is depicted.

Table II. The effect of a previous vaginal delivery on a subsequent trial of labor

	No. of prior vaginal births					
	None			≥ 1		
		With vaginal delivery			*With vaginal delivery*	
Time of vaginal birth	*No.*	*n*	*%*	*No.*	*n*	*%*
Prior to cesarean section (n = 721)	552	443	80	169	144	85
After cesarean section (n = 719)	600	473	79	119	112	94*

*$p < 0.01$.

section was planned. However, the best approach at resolving this issue would be to contrast a group of prior cesarean section patients undergoing a trial of labor with a group of patients wthout a uterine scar. This study is currently underway.

Perinatal outcome. There were seven fetal deaths and nine neonatal deaths in the entire study population for a perinatal mortality rate of 13/1000 births. This perinatal death rate is less than the institutional rate of 18/1000.

Of the seven fetal deaths, six occurred during the antepartum period and one occurred during the intrapartum period. The single intrapartum fetal death happened in a patient at 22 weeks' gestation who subsequently was delivered of a 540 gm stillborn breech infant.

Four of the six antepartum deaths occurred with no uterine dehiscence at 35, 40, 41, and 42 weeks of gestation. All four of these patients were permitted a trial of labor with three delivered vaginally and the fourth undergoing repeat cesarean section after an arrest of dilatation with the delivery of a 4350 gm infant. The cause of the fetal deaths in these four patients was unexplained.

In two additional cases, fetal death was associated with a major uterine rupture. The details of these cases are presented under the Dehiscence subheading. It should be noted that neither of these fetal deaths was associated with a trial of labor and both were judged to be preterm.

Of the nine neonatal deaths, five occurred in infants that weighed <700 gm at birth. The four neonatal deaths in larger infants were all associated with multiple anomalies and included an infant with trisomy, and anencephalic infant, one with a single ventricle and transposition of the great vessels, and one with multiple congenital anomalies. Two underwent an elective repeat section, and two had a trial of labor. In the latter two patients, one underwent repeat cesarean section during an attempted induction as a result of the occurrence of fetal bradycardia, and the other was delivered vaginally of an anencephalic infant.

In summary, nine of the 16 perinatal deaths occurred preterm by gestational age or birth weight. Six of these deaths occurred in overtly immature infants as judged by birth weights <700 gm.

Seven gestations were judged term with four deaths attributable to congenital anomalies. Three unexplained term fetal deaths represent a corrected perinatal mortality rate of 2.5/1000.

Maternal outcome. Some of the factors relating to maternal outcome are listed in Table I. It may be noted that the hospital stay associated with vaginal delivery was 2.3 days compared to 4.3 in those patients who underwent cesarean section.

Standard febrile morbidity was significantly less in patients achieving vaginal delivery than in patients undergoing cesarean birth ($\chi^2 = 85$). When patients undergoing cesarean birth following a trial of labor were compared with those in the group without a trial of

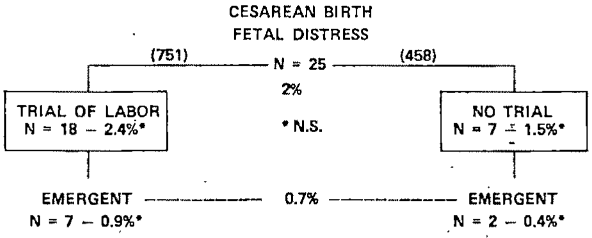


Fig. 7. Cesarean section intervention for all patients is presented. The overall need for an emergency procedure was 0.7%.

labor, the incidence of febrile morbidity was significantly higher ($\chi^2 = 6.26$). However, when the entire trial of labor group was compared with those not undergoing a trial, the febrile morbidity was significantly less ($\chi^2 = 27$) in the trial of labor group.

Dehiscence. The total occurrence of uterine scar separation was 2.4% (Table I). The incidence of dehiscence following vaginal delivery was comparable to that found at cesarean section. The incidence of dehiscence was no greater for those patients having undergone a trial of labor with failure than for those receiving no trial. Further subgroup evaluation failed to reveal any significant differences, that is, in oxytocin usage, previous indication for cesarean section, or number of prior cesarean sections.

In order to better define the significance of uterine dehiscence, cases with this complication were analyzed in detail and divided into two groups. Those cases with a uterine scar separation that required operative intervention were termed uterine rupture. The five cases that were considered uterine rupture are as follows:

CASE 1. A 32-year-old, para 4 woman presented with abdominal pain at 28 weeks' gestation with three prior cesarean sections. Evaluation of the patient demonstrated fetal death. At laparotomy, the fetus and placenta were found in the abdominal cavity, and a complete rupture of a previous classical incision was noted. The weight of the fetus was 2800 gm and the rupture was repaired. The patient had an uneventful postoperative course.

CASE 2. A 22-year-old, para 1 woman presented at 32 weeks' gestation complaining of abdominal pain. The patient was hypotensive and fetal death was diagnosed. Exploratory laparotomy demonstrated that the fetus and placenta had been expelled into the abdominal cavity. The fetus weighed 2160 gm and a uterine rupture had occurred through a prior fundal incision. The wound was closed and the patient had an uneventful postoperative course.

CASE 3. A 28-year-old, para 2 woman presented in labor at term with an unknown type of previous scar. The patient had an uneventful trial of labor and was delivered of a 3700 gm infant with Apgar scores of 9 and 9. Because of a retained placenta and persistent postpartum bleeding, an exploratory laparotomy was performed. A uterine rupture was found in the fundus extending from one round ligament to the other and evidence of a placenta accreta was apparent. The wound edges were resected, then reapproximated and closed. The patient had a benign postoperative course.

CASE 4. A 21-year-old, para 1 woman with a prior cesarean section for cephalopelvic disproportion presented at 42 weeks' gestation in active labor. The patient received oxytocin augmentation for inadequate uterine activity and subsequently had an arrest of dilatation at 5 cm. The patient was taken to the operating room for a repeat cesarean section with an examination revealing no further progress and, as per usual practice, an attempt to elevate the head was performed. At cesarean delivery the head and shoulders had been expelled through a low transverse uterine rupture. Apgar scores were 2 and 9 and the birth weight was 4260 gm. The uterine wound was closed in the usual fashion. The maternal and neonatal hospital courses were benign.

CASE 5. A 19-year-old, para 1 woman with a prior cesarean section for cephalopelvic disproportion presented in labor at 39 weeks' gestation. During the trial of labor severe variable decelerations were encountered. Normal scalp pH values were obtained. An emergency cesarean section was subsequently performed for a persistent fetal bradycardia. At cesarean section, the umbilical cord had prolapsed through a complete low transverse uterine rupture. The infant weighed 3240 gm and had Apgar scores of 2, 6, and 7. The maternal and neonatal postoperative courses were uncomplicated.

Hysterectomy. Seven hysterectomies (0.6%) were performed in the entire study group. Of these, two hysterectomies were performed in patients who had failure of a trial of labor. At cesarean section, both patients had intact scars; hysterectomy was necessary for hemorrhage and atony. There were five hysterectomies performed in patients who had a planned cesarean section. One occurred in a patient undergoing an elective repeat cesarean section with the findings of a complete scar separation and placenta percreta. In the four remaining cases the indications were hemorrhage secondary to laceration extending into the vagina at the time of cesarean section in one, placenta accreta in one, and hemorrhage and uterine atony in two. No patient who was delivered vaginally underwent a hysterectomy.

Comment

The ever-increasing number of patients having undergone a prior cesarean section presents a challenge in an institution such as ours regarding management of subsequent deliveries. An active approach offering a trial of labor based on the confidence that such a course is relatively safe seems to provide benefits to the patient group as a whole. Our confidence in offering a trial of labor based may well be reflected in the unusually high acceptance rate (92%) in our population who were candidates for a trial of labor in contrast to that experienced by others.[8]

It would appear that the indication for a prior cesarean section bears little influence as it relates to the success of achieving a vaginal delivery in a current pregnancy. Patients having had a diagnosis of cephalopelvic disproportion achieved vaginal delivery 77% of the time. It would thus appear reasonable to permit virtually all requesting patients, who qualify, to have a trial of labor irrespective of the previous indication for cesarean section.

Factors that are associated with an enhanced probability of vaginal delivery are a prior vaginal delivery and breech presentation as the prior indication for cesarean birth.

The use of oxytocin in patients wishing a trial of labor must still be further assessed. One must caution that administration of oxytocic agents must be precise via the use of infusion pumps and coupled with careful electronic monitoring of the fetus and mother. The major complication, uterine rupture, in a patient who had received oxytocin (Case 4) may have been related to the obstetric manipulation prior to cesarean section but the association with oxytocin administration cannot be ignored.

The apparent dehiscence incidence was higher at cesarean section than following vaginal delivery. This is likely related to the inaccuracy of diagnosis following a vaginal delivery as opposed to the direct visualization at laparotomy. The dehiscence rates at cesarean section were no different in patients who had undergone a trial of labor as compared to those in whom the procedure was done electively. Evaluation of the subgroups of patients with dehiscence for factors such as oxytocin administration, previous diagnosis of cephalopelvic disproportion, and a previous repeat cesarean section failed to reveal a different rate of dehiscence. Dehiscences found at cesarean section were routinely repaired. The 11 dehiscences found following vaginal delivery were not repaired with the exception of Case 3 in which there was a uterine rupture.

The uterine ruptures noted in the case presentations were associated with prior fundal or classical incisions in three of the five circumstances. We would thus recommend that patients with known fundal (classical) incisions not be permitted a trial of labor. Even so, two of these three patients had preterm uterine rupture. The etiology of such ruptures remains obscure, and subsequent pregnancy management presents a dilemma. The third fundal rupture was probably the result of attempts to remove a retained placenta following a successful vaginal delivery.

The questions relating to subsequent pregnancy management following the observation of a dehiscence or low-segment defect after vaginal delivery remains an open question. Our policy has been not to repair such defects unless significant bleeding is encountered. We are currently attempting to evaluate pregnancy management and outcome in such patients.

Further evaluation of a trial of labor in patients who have had more than one previous uterine incision and low vertical uterine incisions is indicated. Total experience in our institution numbers about 50 patients with no serious complications in this limited experience. The continued evaluation of low vertical scars is of increasing importance with the increasing trend to deliver very low–birth weight infants by cesarean section through a vertical uterine incision which often extends beyond the lower segment.

Perinatal mortality and morbidity occurred in our study population. Three of the fetal deaths in this study group would probably have been prevented by routine elective cesarean section had the procedure been done at 39 weeks. This study does not attempt to compare the outcome of elective repeat cesarean section at 39 weeks versus an approach endorsing a trial of labor. From the standpoint of fetal risks, a trial of labor will most likely be associated with some increase in fetal morbidity. However, this morbidity is not likely to be increased over that of any other group of laboring women. Nonetheless, more study is needed in this area to adequately define the risks to the fetus.

The topic of the occurrence of fetal distress and the need for emergency intervention is one of major concern. The incidence of fetal distress during a trial of labor and emergency delivery for this condition was not statistically different from that occurring in the group in whom repeat cesarean delivery was planned and who had been permitted to enter "early labor." Additionally, the occurrence of fetal distress in the group of patients having had a prior cesarean section for fetal distress was not increased.

In view of these findings one may question if women undergoing a trial of labor truly require more immediate availability of extensive obstetric services than women without a uterine scar. One might ask what services should be available in "every modern obstetric unit"?[11]

The overall febrile morbidity in the group undergoing a trial of labor, including failures, was lower than in the group having cesarean birth. The febrile morbidity in patients having a failed trial was significantly higher than in those undergoing elective repeat cesarean section. However, it was equivalent to that in the group of women having primary cesarean birth in our institution.

It would appear that the major complications and adverse events within this study population all having had a prior cesarean birth were not definitively associated with the process of trial of labor. We thus feel confident that the continuation of this approach within our institution is justified. Specific areas of future investigation will include the option of trial of labor in patients with more than one prior uterine incision, the further evaluation of oxytocin administration, and an evaluation of permitting a patient with a previously known low vertical scar to undergo a trial of labor. In addition, it will be of interest to evaluate subsequent delivery management and outcome in patients who have had a known dehiscence, following vaginal delivery, which was not repaired.

In summary, an active program for trial of labor with certain exclusions and within our facility seems acceptably safe. Our overall success rate is similar to that of others[8, 9] even though our inclusion criteria were much more liberal. A trial of labor approach decreases the total number of cesarean births, resulting in a less expensive and shorter hospital stay. Additionally, the resources of the blood bank, nursing care, and anesthesia services are economized. Future studies will hopefully define better the relative benefits and risks with an expansion of this approach.

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The role of ultrasound assessment of amniotic fluid volume in the management of the postdate pregnancy

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Antepartum assessments of amniotic fluid volumes and their relationship to nonstress test patterns and pregnancy outcomes were retrospectively analyzed in 234 postdate pregnancies. The incidence of clinical oligohydramnios and a nonstress test revealing fetal heart rate deceleration or bradycardia was found to increase as the sonographic estimates of the amniotic fluid volume decreased. Furthermore, the postdate pregnancy with sonographic evidence of an adequate amniotic fluid volume had a significantly better perinatal outcome than the pregnancy without an adequate fluid volume. These results suggest that the postdate pregnancy with evidence of reduced amniotic fluid volume should be considered for a trial of labor with continuous electronic fetal monitoring. (AM J OBSTET GYNECOL 1985;151:304-8.)

Key words: Ultrasound, amniotic fluid, pregnancy

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Biophysical techniques for assessment of fetal status have made major contributions to the reduction of perinatal morbidity and mortality during the past decade. Initially, intrapartum fetal heart rate monitoring[1] provided important insights into the fetal condition, and antepartum fetal heart rate monitoring was developed from these observations.[2] While these techniques continue to aid the clinician, ultrasonography has enabled us to assess more effectively the fetus and its intrauterine environment. As a result, the uses of ultrasonography have grown considerably and have been extended to include the evaluation of fetal status.[3]

With the development of the fetal biophysical profile,[4] antepartum fetal heart rate testing and real-time ultrasonography have been integrated into a single dynamic assessment of fetal well-being. An important component of the profile has been the defining of amniotic fluid volume and its relationship to fetal growth and development. For example, decreased amniotic fluid volume has been shown to be a reliable predictor of intrauterine growth retardation and clinical oligohydramnios.[5]

The relationship between ultrasound estimates of the amniotic fluid volume and the fetal heart rate pattern has not been previously described. However, recent evidence by Platt et al.[6] would suggest a sizable increase in perinatal morbidity in high-risk pregnancies found to have ultrasonic evidence of a reduced amniotic fluid volume. We thus investigated whether pregnancies without an adequate amniotic fluid volume would be more susceptible to umbilical cord compression[7] and would result in an increased incidence of variable decelerations during antepartum fetal heart rate testing. We therefore retrospectively reviewed the pregnancy outcomes and antepartum fetal heart rate patterns for postdate pregnancies with sonographic assessments of amniotic fluid volume at the Los Angeles County/University of Southern California Medical Center between July 1, 1980, and Jume 30, 1981.

Material and methods

The records of patients with postdate pregnancies who underwent biophysical testing in the Fetal Assessment Unit of the Medical Center and were delivered within 7 days of their last evaluation were included in this report. During this period the management of the postdate pregnancy was primarily in accordance with the previous report by Yeh and Read.[8] Biophysical assessment of fetal status was made with the use of the nonstress test and the fetal biophysical profile.

Guidelines for the performance, interpretation, and management of the nonstress test at our institution have been previously described.[6] Patients with fetal heart rate bradycardia revealed on the nonstress test, as described by Druzin et al.,[9] were evaluated for delivery. As part of the current investigation, fetal heart rate decelerations were defined as a decline in the fetal heart rate of ≥ 15 bpm lasting 15 seconds in response to fetal movements. Fetal heart rate decelerations in association with uterine activity were interpreted in the usual manner. Decelerations that immediately followed an acceleration were excluded.[10]

Amniotic fluid volume measurements were based on assessments of the amniotic fluid volume by use of linear-array real-time B-scan ultrasonography. Fluid throughout the cavity with the largest pocket >1 cm in its vertical diameter was considered an adequate amniotic fluid volume or norm (group 1). During the study period a group of patients were identified who had adequate fluid by quantitative measurements (>1 cm pocket), but the overall impression of the sonographer was that the fluid was decreased for the postdate population. This latter assessment of amniotic fluid volume was referred to as adequate but decreased[6] (group 2). Absence of fluid throughout the cavity and a single amniotic fluid pocket of ≤ 1 cm characterized the group considered decreased[5] (group 3). All amniotic fluid volume determinations were done by specially trained antepartum nurses. When it was possible, abnormal scans were confirmed by the obstetric attending staff. During the study period, postdate patients with a decreased amniotic fluid volume (group 3) were considered for delivery; however, like groups 1 and 2, they were managed according to their nonstress test results.

Pregnancy outcome was measured by the incidence of the following: fetal distress that required cesarean delivery, meconium passage, Apgar scores of <7 at 1 and 5 minutes, postmaturity syndrome, and oligohydramnios.

Postmaturity, as determined by the clinical evaluation by the neonatologists, was indicated when either of the following groups of criteria was noted: (1) neonatal examination indicating signs of postmaturity, such as dry and peeling skin, loss of subcutaneous tissue, and long nails, or (2) Rohrer's ponderal index (100 × weight in grams/length in centimeters)[3] of <2.3 and meconium-stained fluid.

Oligohydramnios was based on the clinical estimates of the quantity of amniotic fluid at the time of amniotomy and/or delivery as recorded in the hospital records.

Statistical analysis was done with the use of χ^2 analysis and Student's *t* test. Significance was considered with $p < 0.05$.

Results

During the study period 236 postdate pregnancies were evaluated and a total of 501 fetal biophysical profiles were completed for these patients. In 191 pregnancies (81%), sonographic evidence of an adequate amniotic fluid volume was found in 428 examinations (85%). In the last examination of 45 patients the amniotic fluid volume was described as adequate but decreased in 38 (84%) and decreased in seven (16%). Based on the last sonographic estimates of the amniotic fluid volume, the incidence of antepartum fetal heart rate tests with fetal heart rate decelerations

Table I. Relationship between amniotic fluid volume and pregnancy outcome in 236 postdate pregnancies

	Amniotic fluid volume					
	Adequate (group 1)		*Adequate but decreased (group 2)*		*Decreased (group 3)*	
	No.	*%*	*No.*	*%*	*No.*	*%*
No. and percent	191	81	38	16	7	3
Cesarean delivery	31	16	11	29	3	43
Cesarean delivery for fetal distress	4	2	6	16*	3	43
Meconium	71	37	23	61*	5	71
Oligohydramnios	13	7	11	29*	6	86†
Apgar score <7						
1 minute	31	16	12	32†	6	86†
5 minutes	5	2	1	3	2	29
Birth weight >400 gm	46	24	6	16	0	0
Postmaturity	28	15	11	29	1	14
Bradycardia[9]	4	2	4	11†	1	14
Deceleration or bradycardia	40	21	17	45*	5	71
Birth weight (gm)	3632 ± 529		3503 ± 477		3138 ± 259	
Placental weight (gm)	679 ± 143		682 ± 158		673 ± 178	
Profile-to-delivery interval ≤2 days	39	20	18	47	7	100

*$p < 0.01$.
†$p < 0.05$.

and/or bradycardias was inversely proportional to the sonographic estimates of the amniotic fluid volume: group 1, 21%; group 2, 44.7%; group 3, 71%. A similar but less dramatic trend was observed with respect to the incidence of nonreactive nonstress testing (group 1, 11.6%; group 2, 21.0%; group 3, 33.3%). Finally, the incidence of clinical oligohydramnios correlated with the sonographic estimates of the amniotic fluid volume: group 1, 6.8%; group 2, 29.0%; group 3, 86%.

Pregnancy outcomes for the three groups are illustrated in Table I. The groups were comparable with respect to birth and placental weights. However, group 3 was found to have the lowest mean weights. With the exception of 5-minute Apgar scores, groups 2 and 3 had a significant increase in perinatal morbidity when compared with group 1. There were no perinatal deaths. If group 1 is excluded and groups 2 and 3 are compared, group 3 had a significantly greater ($p < 0.05$) incidence of oligohydramnios and 1-minute Apgar scores <7 and the least favorable outcome.

Cesarean delivery for fetal distress was significantly more common in groups 2 and 3 (Table I). The reason for the fetal distress was primarily fetal heart rate bradycardias, either alone or in combination with other fetal heart rate deceleration patterns. Cesarean delivery for late decelerations, either alone or in combination with a fetal heart rate bradycardia, was necessary in four of 13 instances (30.8%). For the three groups the overall incidence of late decelerations that required cesarean delivery was as follows: group 1, 0.5%; group 2, 5.2%; group 3, 16.7%. On a percentage basis, presence of meconium-stained amniotic fluid was inversely proportional to the amount of amniotic fluid (Table I). Although meconium aspiration was greater in groups 2 (10.5%) and 3 (16.7%) than in group 1 (7.3%), these results were not statistically different.

During the study period, two fetal deaths occurred for an overall incidence in this postdates population of 0.8%. One death occurred in group 1 (1 of 191 or 0.5%) and the other in group 3 (1 of 7 or 14%). In the patient from group 1 with an adequate amniotic fluid volume, the last two nonstress tests were reactive and exhibited fetal heart rate decelerations. Later the same day of the last nonstress test, fetal death was diagnosed. At delivery, a 3900 gm stillborn infant was delivered vaginally and found to have a true knot in the cord. No autopsy was performed. In the second case, the patient had evidence of a decreased amniotic fluid volume on five consecutive visits. The nonstress test was reactive and the fetal biophysical profile score was 8 at each visit except one. At that time, she had a reactive nonstress test with a single late deceleration and a biophysical profile score of 8. Two days after the last fetal evaluation (a reactive nonstress test and decreased amniotic fluid), fetal death was noted. She was subsequently delivered of a 2600 gm stillborn infant. A velamentous insertion of the cord was recorded. No autopsy was performed.

The most dramatic observation predicting an adverse outcome was seen in group 3, the group with decreased amniotic fluid volume, which occurred in seven patients (3.0%). The pregnancy outcomes for this group are also illustrated in Table I. Three patients required cesarean delivery because of intrapartum fetal distress. For these three patients the intrapartum fetal heart rate pattern that necessitated operative intervention was a persistent fetal heart rate bradycardia. However, one of the three patients had late decelerations that antedated the bradycardia. The other two patients did not exhibit late decelerations. Despite a high predictive accuracy of oligohydramnios (86%), only one infant had evidence of postmaturity syndrome.

Comment

Real-time ultrasonography has been advocated as a reliable technique for the detection of oligohydramnios[5] and postmaturity.[12] In the current investigation we expanded our earlier sonographic criteria[5] for the diagnosis of oligohydramnios to include those postdate pregnancies that had adequate fluid by the criteria of Manning et al.[5] but in which amniotic fluid volume was subjectively decreased. Sonography for this group of patients, in whom the fluid was previously described as adequate but decreased, was found to be a fairly reliable predictor of the amniotic fluid volume and postmaturity.

Clinical reliance on this subjective assessment of amniotic fluid volume could lead to numerous clinical problems. For example, by this clinical approach, 17 of 30 cases (57%) of clinical oligohydramnios were accurately predicted, but this positive predictive accuracy was at the expense of specificity. The false positive rates for the groups with decreased amniotic fluid volume (group 3) and adequate but decreased amniotic fluid volume (group 2) were 14% and 71.0%, respectively. The high false positive rate in group 2 may be due to the failure to quantify the sonographic estimates at the time of testing or to record accurately the amniotic fluid volume at the time of amniotomy or delivery by the house staff. At the same time the failure to detect oligohydramnios in the group with adequate amniotic fluid volume (6.3%) could possibly be related to the longer profile-to-delivery interval in this group. As a result, more amniotic fluid could have been reabsorbed, which might have been detected by more frequent assessments of the amniotic fluid volume.

With regard to antepartum prediction for pregnancies with evidence of postmaturity, Rayburn et al.[11] have suggested that a decreased amniotic fluid volume was a reliable predictor. In their series, 83% of the postdate pregnancies with this sonographic finding resulted in delivery of a postmature infant. This is in marked contrast to the current study in which only one of seven patients (14%) with decreased amniotic fluid volume were delivered of an infant with evidence of postmaturity. Although inclusion of a larger amniotic fluid volume did enhance the predictive accuracy of whether an infant was postmature, there did not appear to be a consistent relationship between evidence of postmaturity in an infant and the sonographic estimation of the amniotic fluid volume.

Nevertheless, real-time ultrasound assessment of the amniotic fluid volume was a fairly reliable predictor of the quantity of the amniotic fluid and fetal status. For example, in previous reports that dealt with decelerations on the nonstress test,[12] it had been hypothesized that the amniotic fluid volume and the position of the umbilical cord were largely responsible for the higher incidence of these fetal heart rate decelerations. In the postdate pregnancy, the infant is frequently larger and there is less amniotic fluid as the pregnancy advances.[13] As a result, the umbilical cord is not able to readily escape compression or impingement during a fetal movement or a uterine contraction. In the current investigation, the frequency of nonstress tests demonstrating decelerations and bradycardias increased as the sonographic estimates of the amniotic fluid volume declined. This finding is in agreement with Eden et al.[14] and suggests, as pointed out by these investigators, that if decelerations are seen on a nonstress test, amniotic fluid volume assessment may disclose a reason for the fetal heart rate pattern. In addition, the higher incidence of cesarean deliveries for intrapartum fetal distress because of bradycardias and severe variable decelerations further amplifies the vulnerable cord situation for the fetus when there is evidence of reduced amniotic fluid volume. Lastly, in the patient undergoing a trial of labor, an association existed between the quantity of amniotic fluid and the incidence of intrapartum late decelerations that necessitated cesarean delivery. While this suggests a dual mechanism for oligohydramnios, it magnifies the importance of close observation of the patient with suspected oligohydramnios during labor.

When sonographic estimates of the amniotic fluid volume were used as a predictor of fetal well-being in the postdate pregnancy, patients with evidence of adequate amniotic fluid volume had a significantly more favorable outcome, whereas those patients with the least amniotic fluid volume on ultrasound had the greatest incidence of perinatal morbidity and mortality. As a consequence, patients with the finding of decreased amniotic fluid volume, irrespective of nonstress test results, should be considered for a trial of labor. This approach is supported by the work of Platt et al.[6] in a large group of high-risk pregnancies.

In summary, real-time ultrasound estimates of adequate amniotic fluid volumes in postdate pregnancies were a reliable predictor of fetal well-being. Additionally, we found that the lower the estimated amniotic fluid volume, the greater the incidence of antepartum fetal heart rate tests indicating decelerations or bradycardias. Based on the evidence provided in this investigation, the postdate pregnancy with decreased amniotic fluid volume is at a significant risk for perinatal morbidity and mortality. Therefore, postdate pregnancies with this sonographic finding, irrespective of nonstress test results, should be considered for delivery with a trial of labor and continuous fetal monitoring. While the current sonographic criteria for defining oligohydramnios in the postdate pregnancy were fairly reliable, further investigation, which is currently under way, is required to more accurately define a normal fluid volume in these patients. Until better sonographic criteria for oligohydramnios are established, postdate pregnancies that fall into group 2 should be managed in accordance with the clinical findings and the results of these fetal surveillance tests.

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The accuracy of estimated gestational age based on ultrasound measurement of biparietal diameter in preterm premature rupture of the membranes

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The management of patients with preterm premature rupture of the membranes is often influenced by the ultrasound measurement of biparietal diameter. The cephalic index has been used in pregnancies with intact membranes to predict the presence of an abnormal biparietal diameter that may be unreliable in estimating gestational age. This study analyzed 100 patients with rupture of the membranes between the beginning of gestation week 28 and the end of week 34. The cephalic index was found to be abnormal 45% of the time; all were in the brachiocephalic range. The biparietal diameter was affected by presentation and presence or absence of amniotic fluid ($p < 0.05$). Use of the biparietal diameter was found to be unreliable in estimating gestational age with preterm premature rupture of the membranes. Use of the cephalic index did not enable prediction of which biparietal diameters would be abnormal; therefore use of the biparietal diameter or the cephalic index in preterm premature rupture of the membranes is not recommended. The use of the head circumference, abdominal circumference, and femur measurements may be a better way of predicting gestational age in this select population. (AM J OBSTET GYNECOL 1985;151:309-12.)

Key words: Preterm premature rupture of the membranes, cephalic index, biparietal diameter

Preterm premature rupture of the membranes complicates approximately 2% to 4% of all pregnancies. The management of these pregnancies is predicted on an accurate assessment of gestational age and estimated fetal weight. Normal regular menses, early pregnancy test, early pelvic examination, auscultated fetal heart rate at 19 to 20 weeks, and ultrasound studies are all helpful in determining the estimated date of confinement. Despite the best efforts of physicians to have excellent dating criteria in every patient, all too frequently the gestational age is not known. The combination of poor or unknown dates and preterm premature rupture of the membranes occurs commonly. Obstetric decisions profoundly affect the morbidity and mortality of pregnancies considered premature (<36 weeks), and most obstetric therapy and intervention is based on the physician's assessment of gestational age. Paul et al.[1] found that perinatal mortality was dependent on fetal weight estimated by the obstetrician. If the estimated weight was less than the actual weight, the outcome was significantly worse than when the estimated fetal weight was more than the actual fetal weight.

The most frequently used measurement for ultrasound estimate of gestational age is the fetal biparietal diameter. This measurement may be inaccurate in estimating gestational age when the pregnancy is complicated by preterm premature rupture of the membranes. Wolfson et al.[2] studied patients at gestational ages from 26 to 40 weeks with both rupture of the membranes and with intact membranes. Gestational age estimated by biparietal diameter in the group with rupture of the membranes differed significantly ($p < 0.01$) from the group with intact membranes. An inaccurate measurement of biparietal diameter may lead to an incorrect assessment of gestational age and estimated fetal weight, which then would be used as an inaccurate basis of obstetric management decisions.

Hadlock et al.[3] proposed the use of the cephalic index to screen the biparietal diameter measurement for possible errors due to abnormal head shape affecting the biparietal diameter, but they confined their observations to pregnancies with intact membranes. The purpose of this study is to apply use of the cephalic index to patients with preterm premature rupture of membranes to determine the accuracy of the biparietal diameter prediction of gestational age under these conditions. Fetal presentation, amniotic fluid volume, the presence or absence of labor, and the duration of rup-

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ture of membranes prior to admission were examined for any effect on the biparietal diameter or cephalic index.

Material and methods

One hundred six patients with preterm premature rupture of the membranes between 28 and 36 weeks' gestational age constitute the study population. These patients were taken from a study of preterm ruptured membranes reported by Garite et al.[4] The gestational age of these patients was between the beginning of the week 28 and the end of the week 34. Ruptured membranes was diagnosed by sterile speculum examination, which confirmed pooling of fluid in the posterior fornix, alkaline pH by Nitrazine paper and ferning. Patients with undocumented rupture of membranes or equivocal results were excluded from the study. Six patients were removed from the study population because of incomplete data.

All patients had an ultrasound examination within 4 hours of admission to the hospital. All had good quality measurements of biparietal diameter (BPD) and occipital frontal diameter (OFD). A Picker 80-L B scanner and a Toshiba SAL-20 real-time linear-array ultrasound were used for all ultrasound examinations. The biparietal diameter was measured from the outer margin to inner margin of the skull table, and the occipital frontal diameter was measured from the outer margin to outer margin of the skull table. Both measurements were made at the level of the cavum septi pellucidi or rostrum of corpus callosum and choroid plexus in the atrium of the lateral ventricle, as described by Hadlock et al.[5]

Criteria met by the 100 patients remaining in the study included that clinical dating criteria was good, that the neonatal estimate of age as determined by the Dubowitz score after delivery was within 2 weeks of the clinical dates, and that the patient was delivered within 4 days of the ultrasound examination.

Amniotic fluid volume was considered normal if a pocket of fluid ≥2 cm in the vertical plane was seen anywhere in the uterus. Presentation was either vertex or breech; three transverse lie presentations were excluded from this analysis because these small numbers could not be statistically analyzed. Labor was defined as regular palpable uterine contractions occurring at a frequency of three in a 10-minute period, lasting for at least 1 hour. Duration of rupture of the membranes before admission was divided into three categories: 0 to 4 hours, 4 to 12 hours, and >12 hours. The cephalic index was calculated according to Hadlock et al.[3] (also Hadlock, personal communication) (BPD + 3 mm/OFD × 100) with a normal range for the cephalic index for this study being 70% to 86%, which represents 2 SD from the mean in their sample. The head circumference was measured in the same plane as the biparietal diameter and occipital frontal diameter. The formula used to calculate the circumference was (BPD + 3 mm + OFD) × 1.57 (Hadlock, personal communication). The data were subjected to statistical analysis to determine the nature of variability in the sample using *t* test, χ^2, analysis of variance, and linear regression as appropriate.

Results

The mean gestational age from the last menstrual period was 32.3 weeks with a range of 28 to 36 weeks. An abnormal cephalic index was calculated in 45 patients. Of the 45 patients with an abnormal cephalic index, all 45 had a cephalic index greater than 86% and were considered brachiocephalic. Of the brachiocephalic fetuses, 39 were vertex, four breech, and two transverse. Fetal position was found to be 79 vertex (79%), 18 breech (18%), and three transverse lie (3%). Adequate amniotic fluid volume (≥2 cm) was present in 74 (74%) and absent in 26 (26%). Labor was considered present in 23 (23%) patients, and absent in 74 (75%). In three patients the presence of labor was not coded on the data sheet. The length of rupture of the membranes before admission was 0 to 4 hours, 33 patients; 4 to 12 hours, 33 patients; >12 hours, 30 patients.

Biparietal diameter, occipital-frontal diameter, cephalic index, and cranial circumference were analyzed by *t* test according to presentation (breech versus vertex), amniotic fluid (absent versus present), labor (yes versus no), and duration of rupture of membranes prior to admission to determine whether significant differences existed between groups. The only tests showing a significant difference in means were biparietal diameter by presentation (Table I) and the presence or absence of amniotic fluid (Table II), with gestational age being equal in both groups.

To determine whether technical problems with imaging or measurement would have produced these results, the mean head circumference at each gestational week in our study sample was compared to published means[6] at similar gestational ages. The mean head circumference data in this study was within ±2 SD of the data published by Hadlock et al.,[6] confirming that our imaging and measurements are accurate.

Comments

Many obstetricians and ultrasonographers use biparietal diameter as a single measure to estimate gestational age. Wolfson et al[2] have suggested that biparietal diameter may be unreliable in the face of preterm premature rupture of the membranes. This has been assumed to be due to the effect of cranial molding caused by oligohydramnios. Also, Hadlock et al.[3] have

Table I. Biparietal diameter by presentation

Presentation	*No.*	*Mean biparietal diameter + SD*	*Significance*
Breech	18	7.7 ± .191	$p < 0.05$
Vertex	79	8.1 ± .073	

Table II. Presence or absence of amniotic fluid

Amniotic fluid	*No.*	*Mean*	*Significance*
No	25	7.8 (±.80)	$p < 0.05$
Yes	72	8.1 (±.60)	

Table III. Relationship of cephalic index to other variables

	Normal cephalic index	*Abnormal cephalic index*	*Significance*
Amniotic fluid			
Normal	39	35	NS*
None	16	10	
Presentation			
Vertex	40	39	NS*
Breech	14	4	
Labor			
Yes	13	10	NS*
No	42	33	
Time from rupture to admission (hr)			
0-4 hr	15	18	NS*
4-12 hr	23	11	
>12 hr	17	13	

*By χ^2.

Table IV. Cases with predicted ages more than 2 weeks greater or more than 2 weeks less than age from last menstrual period according to cephalic index

	Normal cephalic index	*Abnormal cephalic index*	*Significance*
Biparietal diameter > or <2 wk from clinical dates	11	7	NS

shown that by calculating the cephalic index (using the figures <74% for dolichocephalic and >83% for brachiocephalic—1 SD), the reliability of using the biparietal diameter in estimating the gestational age can be improved. They described significant error in determining gestational age when the cephalic index was abnormal. Hohler[9] reported that, using ±1 SD from the mean for normal criteria, 38% of the cephalic index's were considered abnormal; however, when ±2 SD were used, only 14% were judged abnormal. The data presented in this study represent the first application of a reliability test (cephalic index) to a possibly distorted ultrasound measurement (biparietal diameter) in premature rupture of the membranes.

In this group of patients, using ±2 SD from the mean to define the abnormal (<70%, >86%), the cephalic index was abnormal 45% of the time with premature rupture of the membranes in the preterm infant (28 to 34 weeks). All of the cephalic indices that were abnormal were in the brachiocephalic range. Of the brachiocephalic fetuses, 39 were vertex, four breech, and two transverse. This appears contrary to our clinical assessment that with preterm premature rupture of membranes, the fetal head is molded toward an increase in occipital frontal diameter and a decrease in biparietal diameter.

Four variables were examined (vertex versus breech, amniotic fluid versus no amniotic fluid, labor versus no labor, duration of rupture of the membranes prior to admission) to determine their effect on the cephalic index and biparietal diameter. As shown in Tables I and II, presentation and amniotic fluid volume were the only variables to significantly affect the biparietal diameter. Kasby and Poll[7] reported data showing that with intact membranes the breech fetus tends to have a smaller biparietal diameter than a comparable group of babies presenting as vertex. Our data are consistent with this finding. Data in Table III shows that the cephalic index is not affected by the other variables. Table IV shows that an abnormal cephalic index did not predict which biparietal diameters would be either more than two weeks greater or more than 2 weeks less than the clinical and neonatal assessment of gestational age. This amount of error in a measurement should cast doubt on the reliability of use of biparietal diameter even with a normal cephalic index.

These data support the concept that with preterm premature rupture of the membranes, the maternal pelvis tends to mold the fetal head in the vertex position to the brachiocephalic state. This error would tend to cause an estimation of gestional age and estimated fetal weight greater than the actual age.

To demonstrate the predictability of menstrual age within the sample, linear regressions were calculated for cranial circumference and biparietal diameter. While significantly good equations were determined, the amount of variation that they explain is considerably less than that found in the reports of Hadlock et al.[6] and Wolfson et al.,[2] 47% versus 61% to 83% respectively. This illustrates the variability of cranial size within our preterm premature rupture of the membranes sample but is also subject to the effects of sample truncation, to the age range of 28 to 35 weeks of gestation, as noted by Wolfson et al.[2]

It appears (in patients with preterm premature rupture of the membranes) that early in the third trimester, ultrasound estimation of gestational age by biparietal diameter is particularly unreliable. Cephalic index can predict when the biparietal diameter should not be relied on with intact membranes, but in this sample a normal cephalic index was not predictive of a usable biparietal diameter and therefore an inaccurate prediction of gestational age by ultrasound measurements was equally likely with normal cephalic indexes and abnormal cephalic indexes. The biparietal diameter measurements were so variable that, used alone in preterm premature rupture of membranes to determine gestational age, they appeared to be of little help to the clinician.

Hadlock et al.[8] and Hohler[9] have recommended the use of multiple measurements, specifically biparietal diameter, femur length, head circumference, and abdominal circumference to increase accuracy in estimating gestational age in the third trimester. Their conclusions were that with multiple measurements the error observed in assessing gestational age by ultrasound in the third trimester was reduced from ≥ 3 weeks to 2 weeks. With an abnormal cephalic index in preterm premature rupture of membranes, the biparietal diameter should definitely not be used and the other measurements (i.e., femur length, head circumference, abdominal circumference) must be employed in determining gestational age. In the case of preterm premature rupture of the membranes with a normal cephalic index, it also appears that the biparietal diameter should not be used when determining gestational age and that a combination of the other multiple measurements should be employed; therefore the cephalic index in cases of preterm premature rupture of the membranes does not differentiate which biparietal diameter measurements are reliable. Although there is no study in the literature confirming that a multiple measurement technique with preterm premature rupture of the membranes is superior, it would seem reasonable that multiple measurements would decrease the variability and help obtain a more accurate estimation of gestational age.

Neither labor, fetal presentation, amniotic fluid volume, or duration of rupture prior to admission have a predictive effect on the cephalic index or the biparietal diameter measurement, and therefore cannot be used to further define which pregnancies have distorted biparietal diameters. Again, it would be reasonable to assume that the multiple measurement technique would help reduce variability and improve predictability under all conditions.

The findings of this study have important implications in cases of preterm premature rupture of the membranes: (1) estimated gestational age based on biparietal diameter is unreliable; (2) cephalic index is not useful in separating fetuses with normal head measurements from abnormal measurements; (3) other measurements of fetal size, i.e., head circumference, abdominal circumference, and femur length, should probably be relied on to determine gestational age; (4) vertex presentation changed the biparietal diameter, with the estimated gestational age being older than the actual age.

Future studies need to be made to evaluate the multiple measurement technique in preterm premature rupture of the membranes and to confirm that these measurements are reliable in predicting gestational age in this rather select group of patients.

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Current controversies in obstetrics: Wrongful life and forced fetal surgical procedures

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Contemporary reproductive decision-making is characterized by the availability of a distinctly secular set of considerations. In the past it was presumed that sexuality was for reproduction and that there were ways of generally distinguishing morally between natural versus unnatural interferences in reproduction.[1] It was presumed, in addition, that there were compelling state interests that could establish a pronatalist reproductive policy.[2] These, however, no longer appear justifiable. This is not to say that such arguments are simply wrong. Rather, it has been recognized that they cannot be justified in the absence of special religious, metaphysical, or political premises, which premises cannot be generally secured in rational secular terms. Their force and governance must be restricted to special domains defined by communities sharing their presumptions.[3] In contrast to these special moral communities, a general set of secular moral assumptions has been articulated. One must recall here the relatively recent decisions of the Supreme Court in *Griswold v. Connecticut* in 1965, that it is unconstitutional for states to forbid the dissemination of contraceptive information and advice to married persons,[4] and in *Eisenstadt v. Baird* in 1972, which extended access to contraceptive materials to unmarried individuals as well.[5] These decisions were then further augmented by *Roe v. Wade*[6a] and *Doe v. Bolton*[6b] in 1973. The result has been the articulation of a secular ethics which places the locus of reproductive decision-making in the hands of the reproducing couples and ultimately in the hands of the women who could become pregnant.

This secular understanding of reproductive ethics brings a moral tension to discussions (1) regarding the obligation to provide information so that would-be parents can avoid the birth of a defective child and (2) regarding the obligation to impose treatment on behalf of a fetus. First, moral discussions now clearly occur on two levels, one, that of general secular moral arguments, and a second, the level of the religious and cultural commitments that frame our concrete moral lives. In addition, there is a conflict integral to the language of ethics itself. On the one hand, the language of ethics, especially in secular pluralist contexts, unavoidably gives an accent to individual rights of self-determination. In conflict with this autonomy-oriented dimension of ethics are concerns with beneficence, concerns with the best interests of patients. The first, the autonomy-oriented, reminds us of what it means to understand ethics as an alternative to force as a basis of conflict resolution.[7] It contrasts with concerns for the best interests of others, which concerns are consequence-oriented. In the case of competent individuals, they are usually, though not always, held to have the right to choose on their own behalf, even if it is not in their best interest. There is as well a presumption in favor of individuals being the best judges of their best interests. However, in the case of fetuses, where there is no autonomy to respect, moral concern is directed to the best interests of the fetus. This leads to conflicts regarding who can be the best judge of these interests and of how one is to compare the interests of the fetus with the interests of the pregnant woman. The conflict between respecting freedom and securing the best interest of others complicates even further the tensions within morality. These tensions are expressed in obstetrics as physicians attempt to treat both women and their fetuses.

Tort-for-wrongful-life suits

These tensions are displayed in the concern to avoid those pregnancies which could lead to individuals being born with handicaps, deformities, or significant disadvantages, which concern has provided the moral and legal backbone of tort-for-wrongful-life suits. What is at stake are not simply issues of failures of a sterilization procedure or of contraceptive means, or increased costs to the parents, but an injury to the child which often could only have been avoided by that child not coming into existence. For example, as in the case of *Gleitman*

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v. Cosgrove in 1967,[8] Mrs. Gleitman sought recovery on the basis of her not having been informed that many physicians considered an abortion proper after a woman contracted rubella during pregnancy. The New Jersey Supreme Court awarded damages to the parents but not to the infant plaintiff. A similar argument was developed in the case of *Stewart v. Long Island College Hospital*,[9] where suit was filed on the basis that Mrs. Stewart was not fully informed that a number of physicians considered abortion a proper response to the fact that she had contracted rubella during early pregnancy. Though the suit asked for damages to be paid not just to the parents but also to the child, the court awarded damages only to the parents. Such could best be termed a wrongful birth case, in that costs were not awarded to the child, but to the family, to compensate for the costs involved in raising a handicapped infant. (One may wish to distinguish between actions by parents for tort for wrongful birth, based on a child having been born or having been born defective because of negligent treatment or counseling, versus tort for wrongful life actions brought by a child for having been born or born defective. Wrongful conception actions are a species of the former and include cases of failed sterilization or contraception.)

As one enters the 1980s, one finds a number of attempts both by parents to recover damages because they were not able to avoid the birth of a handicapped child whose birth they would have avoided had they not been negligently denied information and by children who argued that their handicaps made life worse than no existence and that the difference between these two states of affairs could be assigned a pecuniary value. (For a review of the issues raised by tort-for-wrongful-life cases, see references 10 and 11.) The first genre of suits by parents was already successful in the 1970s. The second was not. (The holding in *Park v. Chessin*, which, in 1977, affirmed the right for recovery on tort-for-wrongful-life grounds, was reversed.[12])

The matter changes somewhat dramatically in the 1980s with the 1981 ruling of the Court of Appeals of the Second District of California in *Curlender v. Bio-Science Laboratories*.[13] Here the court was faced with the case of the incorrect transmission of information with regard to whether a couple carried Tay-Sachs disease. An affected child was born, and both the parents and the child sued for recovery. The court awarded damages to both. In addition, the court raised the possibility that parents might be liable to their children for damages for "proceeding with a pregnancy" which they knew would likely lead to a damaged offspring. As the court opined, "Under such circumstances, we see no sound public policy which should protect those parents from being answerable for the pain, suffering and misery which they have wrought upon their offspring."[14] The liability of parents was not sustained in another California case, *Turpin v. Sortini*.[15] In addition, the liability of parents was precluded by an act of the California legislature.[16]

However, the general principle that one may be liable to both parents and their children for not having given adequate information that would have influenced reasonable individuals in their choices regarding contraception and abortion remains in place. For example, in a 1983 Washington State decision, *Harbeson v. Parke-Davis*, involving a woman taking Dilantin who argued that she was not adequately informed regarding the possibilities of "fetal hydantoin syndrome," the court found that the parents had a right to prevent the birth of a defective child.[17] The court held that the Harbesons could recover extraordinary educational and medical expenses, in addition to damages for emotional injury. Also, it held that such children could recover special damages to recompense them for extraordinary medical care during their lives. However, the court followed *Turpin v. Sortini* in denying the child damages for pain and suffering.

What can one say about the moral presumptions of these holdings? First, there is the presumption that there is no serious conflict between fetus and parents, at least up until the point of viability, in that up until that point women possess an unchallenged right to terminate a pregnancy. The value of the fetus is subordinated to the wishes of the parents and the rights of fetuses prior to viability are minimal at best. Second, until viability, the fetus possesses rights as a placeholder for a future person. If the fetus comes alive ex utero, it then can sue as a damaged person. There is a contrast between parents and physicians, on the one hand, who are actual persons, and the future persons that the damaged fetuses could become, on the other. This notion of a future person does not require a notion of potentiality, as is raised by some opponents of abortion. Its sense depends rather on understanding that, if an individual plants a bomb under a high school to go off 20 years from now, he would be guilty of murdering the teenagers in the school, even if they had not yet been conceived at the time the bomb was planted. At the time of the murder, the teenagers are actual persons. However, if children are never born to fill the school, no murder occurs. (For a recent overview of the moral issues raised by abortion, see reference 18.) Third, there is no assumption that there is an overriding good involved in bringing a child into the world. There is no moral duty either to conceive such a fetus or to bring such a nonviable fetus to term. The choice to bring a pregnancy to term entails costs for parents. Those who provide insufficient information to parents so that they cannot adequately engage in contraceptive and abortion decisions may be responsible for the costs involved in the care of a handicapped child and for special costs sustained by the child.

In summary, the result of the exploration of these issues in our democratic, pluralist society has been the articulation of a public policy with its own moral assumptions. Prospective parents in general, and women in particular, have come to be recognized as reproductive decision-makers, able and responsible to decide regarding the inception of a pregnancy or its termination. The *Curlender* decision presumes at least a moral obligation on the part of the parents to consider the likely quality of life of the future children they may produce when deciding to initiate and/or continue with a pregnancy. Moreover, physicians who would negligently or willfully fail to provide information for such decisions become morally if not legally liable. This represents a major shift in the understanding of reproductive mores, placing the accent on: (1) the rights of individual freedom in fashioning reproductive decisions within (2) the constraints of responsibility to the future child, if it is conceived and brought to term.

The issue of fetal rights gains salience with fetal surgical procedures and cesarean sections, so that one must ask at what point may one intervene to protect the best interests of the fetus, even over the objections of the mother. Tort-for-wrongful-life cases remind us that, at least in terms of secular moral arguments, the difficulties raised by defective fetuses can be solved by good contraceptive use or by abortion before the fetus is viable. The questions raised by fetal surgical procedures and cesarean sections, in contrast, focus on the consequences of the women's choice to have the pregnancy go to term. The issue is then how the best interests of fetuses, destined to go to term, should be understood as fetal rights.

The emergence of a concept of fetal rights

One might have thought, following *Roe v. Wade* and *Doe v. Bolton*, that the notion of fetal rights would not gain substance. After all, *Roe v. Wade* established the right of women to use abortions despite state laws, even after viability, if the abortion is sought to secure the life or the health of the mother. Indeed, prior to viability, women, with their physicians, were acknowledged as having an unchallenged right to make abortion decisions. But even within the constraints of *Roe v. Wade*, one can ask what are the obligations one has to a fetus, once one has decided not to abort it and once the fetus reaches the point of viability. Does one then have an obligation to protect the fetus' health and well-being insofar as those interventions do not jeopardize the health and well-being of the mother? Again, one must not forget that *Roe v. Wade* allows abortions at any point in order to preserve the life and health of the mother. However, one must also recognize here an extension of the reasoning in *Curlender v. Bio-Science Laboratories*, where the court held that parents could be sued by their children if they were allowed to be born defective. This legal dictum, which is contrary to the traditional doctrine of parental immunity, raises at least the issue of the moral obligation of parents to do what is necessary to secure the health and well-being of a fetus and thus a future person, once they have decided to go through with the pregnancy.

This issue has been joined in the 1981 Georgia Supreme Court holding in *Jefferson v. Griffin Spalding County Hospital Authority*.[19a] The case involved a woman from Butts County, Georgia, with placenta previa in her thirty-ninth week of pregnancy, who, as a member of the Shiloh Sanctified Holiness Baptist Church, refused a cesarean section on religious grounds. The Department of Family and Child Services, acting on behalf of the Georgia Department of Human Resources, petitioned the Butts County Juvenile Court for temporary custody of the fetus. The court took cognizance of the fetus as a deprived child and Jessie Mae Jefferson was ordered to submit to a cesarean section by court order. (*Mirabile factu*, Jessie Mae Jefferson was delivered vaginally without difficulty. See reference 19b.) The decision in this case must be understood in terms of the court having been given evidence that the fetus stood a 99% chance of dying and the woman a 50% chance of dying in the absence of a cesarean section. It was against this background that Justice Hill ruled: "[W]e weighed the right of the mother to practice her religion and to refuse surgery on herself, against her unborn child's right to live. We found in favor of her child's right to live."[20]

The possible range of implications of this and similar court rulings is already being explored by legal scholars in an attempt to determine at what point a woman may be forced to submit to fetal surgical procedures or to cesarean section on the basis of reasoning similar to that developed in the Jessie Mae Jefferson case. (For an exploration of these issues, see reference 21.) From a legal point of view, much remains unclear about what amount of danger to the fetus will justify what amount of pain, discomfort, and risk to the mother. There is a 1983 case of *Taft v. Taft*,[22] in which the husband of a woman in her fourth month of pregnancy attempted to gain a court order forcing her to submit to a cerclage operation to prevent miscarriage. The court refused. One must note, however, that the fetus was not yet viable. Jeffrey Lenow, in his study of the fetus as a patient, offers the case of a 14-year-old girl, 26 weeks pregnant, who experienced premature labor because of a rupture of the amniotic sac.[21] Given the fetus' gestational age, the judgment was that its survival could be maximized only by cesarean delivery. The patient, however, wished a vaginal, not a cesarean, delivery, and her wishes were followed. As Lenow observes, had the woman been in her 30s and interested in having a child, a cesarean section would have been performed. In fact, had she been older she may have been forced, as many women, to submit to a cesarean section.[23] The range of cases from those which tolerated the refusal of a cesarean delivery to those which have invited intervention leave unclear how one is to balance concerns for fetal safety with maternal risks.

Decision-making is rendered even more complex when it appears that the fetus is suffering from serious defects. Some cases can be more easily resolved when the child about to be born is unlikely to meet the minimum conditions for being a moral agent, so that the possibility of doing injury to a future person ceases as well. A somewhat widely accepted example would be that of anencephalic infants. Though these are recognized as persons before the law in certain senses (that is, qualifying the parents for an income tax deduction for a dependent child), they would not count as a person in a strict moral sense or a social sense.[24] This point has been recognized even by theologians with regard to the limits of the duty to treat a defective child.[25] There also should not be a duty to treat fetuses that would be born with defects likely to preclude survival beyond infancy, in that such treatment would not benefit a future person. The issue is relatively clarified when there will not be a future person likely to be injured by the failure to provide maximal safety of delivery through a cesarean section or fetal surgical procedure.

The more troubling moral questions arise in that genre of cases that have become the object of the Baby Doe controversy.[26, 27] There is the question of what protections of cesarean delivery or fetal surgical procedures ought to be afforded to a fetus which will at best develop into a child with serious handicaps. It would be preferable to avoid such conflicts by the use of either contraception or abortion, rather than to have the train of events issue in the question of whether maximally safe means of delivery should be employed for a child likely to be born with significant mental or physical handicaps. Moreover, women should be informed of what are likely to be the choices if they proceed with such a pregnancy beyond viability. In any event, it will not be possible to answer these questions in full without answering the issues raised by the Baby Doe controversy with regard to family sovereignty and the range of disabilities which we will hold to be appropriate for withdrawal of treatment or for less than fully aggressive treatment.

At this point, rather than to explore this second and complex issue, it is enough to recognize two points. First, societies tend to be more accepting of the noninvestment of resources in saving neonates than of their noninvestment in saving older children. (For an examination of attitudes toward infanticide in the ancient world, see reference 28. As to the rather unofficial acceptance of infanticide in recent times, see reference 29.) Second, one will need to recognize that any minimal notion of family integrity will put the moral burden of proof on outsiders to show that families are choosing wrongly rather than on families to show that they are choosing correctly in their decisions regarding the treatment of defective newborn infants. If one enters the domain of the family with state force in order to resolve the conflicts between the decision of the family not to treat and of representatives of the state to treat, the state should at least sustain the costs consequent upon the invasion.

So, also, to set aside an individual's right not to be touched, much less invaded, without permission, one must show at least a duty on the invaded person's part to the care of the fetus under such extreme circumstances. This can be established if abortions are readily available, the woman understands the circumstances to which she would be exposed, and decides to continue with the pregnancy. (I am indebted to Judith Areen of Georgetown University School of Law for a discussion of this point.) Such can usually be reasonably interpreted as a commitment to bringing the fetus safely to term under the conditions known prior to viability. These considerations would give grounds against imposing an experimental form of treatment. A woman cannot reasonably be expected (even with the aid of her physician) to have anticipated such an intervention. At most, one could generally expect that she would have learned about, and taken account of, those treatments which are standard in the sense of generally accepted by the medical community. Second, if, after viability, significant new information becomes available regarding the handicaps of the fetus or the need for a cesarean section or fetal surgical procedure, justifications for invasion of bodily integrity become more difficult. Perhaps a compromise could be found in agreeing that when the state enters the sovereign boundary of an individual's body under such circumstances, the state must be willing to accept all the costs entailed, not only those such as are involved in a cesarean section or fetal surgical procedure but the costs entailed by the treatment and upbringing of a defective child who is saved through such procedures. Third, there should be a high certainty of success from the procedure and a high probability of injury to the fetus in the absence of intervention. The requirement of likelihood of success gives another reason to hold that it would be morally improper to force a woman to submit to an experimental treatment on behalf of her fetus. Finally, the fiduciary duties of the mother to the fetus are surely not absolute. Decisions involving the balancing of ranges of possible harms and benefits introduce sufficient degrees of uncertainty to allow one morally to err within that uncertainty on behalf of the wishes of the pregnant woman. In that the mother is giving the child the gift of life, it seems reasonable that the mother be able to condition that gift upon the mother not being exposed to a significant risk of death or injury or a departure from her ideas of what a rewarding birth process would be (that is, natural childbirth, early opportunity to nurse the child comfortably), unless the risks to the future child are of a serious nature and of a high probability.

If a policy develops of imposing treatment on unconsenting women, we will need to determine what level of risk can justify what level of interference. Such a determination will be necessary in order to give fair warning to women in advance of what may be entailed by their continuing with a pregnancy rather than seeking an abortion. Moreover, the threshold for intervention will need to be fashioned so as not to justify major intervention only on the basis of rather minor or low-probability benefits. The threshold will need to be set somewhere between (1) the rather accepted notion of minor interventions for major risks, as have been associated with the transfusion of pregnant women,[30a, 30b] and (2) recent suggestions that pregnant women with thought disorders not be afforded the option of treatment but instead be committed against their wishes in order to avoid even a low likelihood of third-trimester sequelae from drug treatment.[31]

Conclusions

Where, then, do these reflections lead us? First, they show us that, despite the development of notions of abortion rights through *Roe v. Wade* and *Doe v. Bolton*, the moral status of fetuses is not set aside. In fact, we have been led to a nuanced set of initial distinctions with regard to the moral standing of fetuses. The development of the notion of individual freedom in reproductive decision-making and the availability of greater knowledge concerning reproductive outcomes, along with increased technological capacities to alter such outcomes, have led to increased reproductive responsibility. The very availability of abortion, perhaps paradoxically for some, has increased the plausibility of maternal duties to fetuses in that a woman's continuing with a pregnancy is more of an expression of free choice than was the case in the past. These duties will surely lead to a number of cases in which one will be able successfully to argue that it will be morally permissible to compel a cesarean section and perhaps even fetal surgical procedure. We are only beginning to understand all the moral implications of our technological advances and their implications within the context of a free, pluralist, secular society.

As we shoulder this task, we will most likely find ourselves living in a moral world appreciably more complex than that of the past. In the process, obstetrics will have contributed to the ever-ongoing cultural endeavor of our becoming clearer about ourselves and our moral values. This is, after all, the central human task. This will be a far from mean contribution from medicine to our general culture.

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Nonstressed antepartum cardiotocography in patients undergoing elective cesarean section—Fetal outcome

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In a prospective study of 409 patients monitored with nonstressed antepartum cardiotocography and delivered by elective cesarean section, cardiotocography was requested for 170 because of clinical indications. This group had higher incidences of abnormal cardiotocography ($p < 0.001$), fetal growth retardation ($p < 0.001$) and neonatal deaths ($p < 0.025$) than had the group without such requests, suggesting that clinicians effectively selected the high-risk pregnancy for testing of fetal well-being. Cardiotocographic evidence of critical reserve was found in 17 of 170 patients (10%) tested for a clinical indication and in none of the 239 patients in the control group. Patients with abnormal cardiotocography results had significantly higher incidences of cord arterial blood pH <7.26 ($p < 0.05$) and Apgar scores of <6 at 1 minute ($p < 0.001$), showing that an abnormal cardiotocogram is indicative of a fetus at risk of having hypoxia. (Am J Obstet Gynecol 1985;151:318-21.)

Since the introduction of antepartum cardiotocography at the Mercy Maternity Hospital, Melbourne, in 1973, ever-increasing dependence has been placed on this method of assessment of fetal well-being to aid in selecting the appropriate time for and route of delivery. Studies have attempted to correlate various cardiotocography patterns with parameters of perinatal outcome after either spontaneous or induced labor.[1, 2] The inherent drawbacks of such studies have included the long interval between performing the antenatal cardiotocography and birth of the infant and the effects of labor and drugs on the fetus. To minimize such problems, we examined the relationship between antepartum cardiotocography patterns and perinatal outcome in patients delivered by elective cesarean section.

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Table I. Classification of cardiotocographic patterns

Fetal reserve	*Beat-to-beat variation (bpm)*	*Accelerations per 10 min >15 bpm*	*Late decelerations*
Satisfactory	≥7	≥5	0
Reduced	5-7	<5	0
Critical	<5	<5	Present

Material and methods

The study group, consisting of patients undergoing elective cesarean section, was divided into two subgroups: (1) the "request" group for whom the clinician had previously requested a cardiotocography because the pregnancy was perceived to be at risk either on clinical grounds or because of subnormal urinary estriol excretion and (2) the "nonrequest" group for whom a cardiotocography had not previously been performed. All patients were monitored within 24 hours of cesarean section for at least 20 minutes with the use of a Model 8031A cardiotocogram (Hewlett-

Table II. Prenatal complications, gestation, birth weight, and type of anesthesia administered

	Group of patients			
	Nonrequest (n = 239)	*Request (n = 170)*	*Total (n)*	*P*
Prenatal complications				
Preeclamptic toxemia, hypertension, or chronic renal disease	4	52	56	<0.001
Fetal growth retardation or low urinary estriol assay	3	42	45	<0.001
Poor obstetric history	21	23	44	NS
Diabetes mellitus	–	16	16	<0.001
Antepartum hemorrhage	9	12	21	NS
Diminished fetal movements	–	7	7	<0.01
Weight loss or static weight	–	7	7	<0.01
Postterm	1	5	6	<0.05
Breech or malpresentation	39	4	43	<0.001
Cephalopelvic disproportion	152	–	152	<0.001
Other	10	2	12	NS
Gestation				
≤37 wk	236	129	365	<0.001
34-36.6 wk	2	28	30	<0.001
<34 wk	1	13	14	<0.001
Birth weight				
≤4000 gm	17	11	28	<0.001
2501-3990 gm	220	129	349	<0.001
1500-2500 gm	–	23	23	<0.001
<1500 gm	2	7	9	<0.001
Anesthesia				
General	137	105	242	NS
Epidural	102	65	167	NS

NS = Not significant.

Packard) with a paper speed of 1 cm/min. The cardiotocographies were read by a member of the medical staff of the fetal monitoring unit without knowledge of the patients' subgrouping and were classified as satisfactory, reduced, or critical according to the criteria detailed in Table I. At the time of the operation, details of the type of anesthesia (general or epidural) and the time between incision of the uterus and delivery of the baby were recorded.

Arterial blood was collected immediately after clamping of the cord into a heparinized syringe and transported in ice for acid-base analysis by means of a Model 175 automatic pH/blood gas system (Corning Medical). Statistical analysis was performed by means of χ^2, Fisher's exact probability, and Kolmorgorov-Smirnov two-sample tests where appropriate.

Fetal growth retardation (small for dates) was diagnosed when birth weight was below the tenth percentile according to gestational age for infants born in our institution.

Results

During the period from January, 1981, to December, 1982, 409 patients undergoing elective cesarean section were monitored; 170 were in the request group and 239 in the nonrequest group.

The prenatal complications, gestation, birth weight and type of anesthesia used are listed in Table II. Since the majority of elective cesarean sections in the nonrequest group were performed because of cephalopelvic disproportion or malpresentations, the highly significant difference in the incidences of these complications between the two groups is expected. The main indications for requesting cardiotocography surveillance were hypertensive disorders and intrauterine growth retardation detected either clinically or by subnormal maternal urinary estriol excretion. These disorders occurred in 94 of the 170 (55.3%) request group and in only 7 of the 239 (2.9%) nonrequest group of patients ($p < 0.001$).

The high-risk nature of the pregnancies where monitoring was requested is shown by the increased frequency of premature delivery (24.1% versus 1.3%) and of infants with birth weights of ≤2500 gm (17.6% versus 0.8%) in this group; in each instance these differences were statistically highly significant ($p < 0.001$). These differences are not surprising as the monitored group had a high incidence of maternal conditions that necessitated premature delivery even when the cardiotocographic tracing was normal. Also in many cases the elective cesarean section was indicated by the abnormal cardiotocography.

There was no statistical difference between the utilization of general anesthesia in the two groups, since it

Table III. Incidence of abnormal cardiotocograms and perinatal results in the nonrequest and request groups of patients

Group	No.	*Cardiotocogram*				*Cord blood pH <7.26*		*Apgar score <5 at 1 min*		*Fetal growth retardation*		*Neonatal death*		*Major fetal malformation*	
		Normal		*Abnormal*											
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Nonrequest	239	205	85.8	34	14.2	28	11.7	33	13.8	7	2.9	0	0	9	3.8
Request	170	102	60.0	68	40.0	25	14.7	33	22.4	30	17.6	5	2.9	14	8.2
Significance				p < 0.001		NS*		NS		p < 0.001		p < 0.025		NS	

*NS = Not significant.

Table IV. Perinatal results according to the cardiotocographic pattern

Cardiotocographic pattern	*No.*	*Cord blood pH <7.26*		*Apgar score <6 at 1 minute*		*Fetal growth retardation*		*Neonatal death*		*Major fetal malformation*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Normal	307	33	10.7	39	12.7	18	5.9	1	0.3	12	3.9
Abnormal											
Reduced	85	15	17.6	23	27.1	13	15.3	2	2.4	8	9.4
Critical	17	5	29.4	7	41.2	6	35.3	2	11.8	3	17.6
Total abnormal	102	20	19.6	30	29.4	19	18.6	4	3.9	11	10.8

was used in 61.8% of the request group and in 57.3% of the nonrequest group. Although the incidence of acidosis (cord pH <7.26) was not influenced by the type of anesthesia used (general anesthesia 14.5%, epidural 10.8%) there was an increase in the number of infants with low Apgar scores (<6) at 1 minute when general anesthesia had been administered (22.0%) compared with epidural analgesia (9.6%). This difference was statistically significant ($p < 0.01$).

Difficulty in delivery of the infant and/or uterine incision–to–delivery interval of >3 minutes did not result in a statistically significant increase in fetal acidosis, although the latter was associated with an increase in low Apgar scores ($p < 0.05$).

The perinatal results and incidences of abnormal cardiotocography patterns in the nonrequest and request subgroups of patients are shown in Table III. The differences in the incidences of abnormal patterns, fetal growth retardation, and neonatal deaths were all significant. Although 34 (14.2%) of the nonrequest group had an abnormal cardiotocography pattern, the degree of abnormality was merely reduced in all cases, whereas in the request group 17 of the 68 cases with an abnormal result had a critical reserve pattern (Tables III and IV) ($p < 0.001$).

The perinatal results according to the cardiotocography pattern are also shown in Table IV. The increased incidences of cord pH <7.26 and Apgar score <6 at 1 minute in cases with abnormal cardiotocography patterns (19.6% and 29.4%, respectively) were both significant ($p < 0.05$ and $p < 0.001$, respectively). Although the incidences of both cord pH <7.26 and Apgar score <6 were greater in those with a critical reserve when compared with those with a reduced pattern, these differences did not achieve statistical significance. There was a statistically significant difference in the incidence of fetal growth retardation between the groups with critical (35.3%) and reduced reserve (15.3%) on cardiotocography ($p < 0.05$) and also between the groups with reduced reserve and normal (5.9%) cardiotocography ($p < 0.001$). The single neonatal death in the satisfactory reserve group (Table IV) was due to Potter's syndrome. Three of the four fetuses with an abnormal cardiotocography pattern who died in the neonatal period had major malformations incompatible with life, and the remaining infant, who weighed 944 gm at birth, died 10 days later from the complications of prematurity. Again the increased incidence of neonatal death in those with reduced fetal reserve in contrast with the group with normal cardiotocography was significant ($p < 0.03$).

Comment

The dramatic fall in perinatal mortality in recent years has removed death as a useful index of the quality of obstetric care and has left perinatologists with a lack of reliable and easily applied criteria for use in gauging the results of their management regimens. Apgar scores <6 at 1 minute and cord pH <7.26 are indicators of hypoxia and are used to assess the quality of the infant at birth. Other parameters used are the incidences of growth retardation and major malformations. Both Apgar scores and cord pH measurement have their limitations; the former is subjective and the latter can be influenced by a number of factors, including technical difficulties in collecting and measuring the blood sample and maternal acid-base disturbances.[3, 4] To improve our assessment of the infant at birth, we used the 1-minute Apgar score and cord blood pH status, together with the usual parameters of assessment, i.e., the incidences of growth retardation, major malformations, and perinatal deaths.

This study has shown that the selection of patients into a high-risk category by use of clinical judgment, together with measurement of urinary estriol excretion, was effective, since higher incidences of abnormal cardiotocographic tracings, growth-retarded fetuses, and neonatal deaths occurred in the request group. Moreover, there was no case of critical fetal reserve or perinatal death in the nonrequest group. The 40% incidence of abnormal tracings in the request group was explained by the high-risk nature of the series as well as the fact that the cesarean section was in many cases performed because of the abnormal cardiotocography. This figure compares with the overall incidence of abnormal cardiotocography of 15.7% (1013 of 6438) for all patients monitored antenatally in the hospital between 1973 and 1983. We were further reassured by the finding that the grading of cardiotocographic abnormality (reduced: critical) used in our unit was meaningful in terms of incidences of fetal growth retardation, low cord pH, and low Apgar scores, which increased as the cardiotocography tracing became more abnormal. The only death in the group with satisfactory reserve was due to Potter's syndrome.

The association between lethal congenital malformations and critical fetal reserve patterns (17.6%) emphasizes the necessity to perform an ultrasound study and perhaps also a radiographic examination of the fetus before performing a cesarean section for purely fetal indications. When these tests fail to reveal such an abnormality, the fetus must then be given the benefit of the doubt; otherwise the unfounded assumption that the baby is doomed squanders the whole value of antenatal cardiotocographic surveillance. Indeed, when there is evidence of critical fetal reserve, the quality of survival of infants born by elective cesarean section is satisfactory.[5]

Consistent with other reports, the use of general or epidural anesthesia did not affect cord pH, although there were more low Apgar scores in infants who had been subjected to general anesthesia.[6, 7] The reported association of fetal acidosis and low Apgar scores with prolongation of the interval between incision of the uterus and birth of the infant[8] was examined and only an effect on the Apgar score was confirmed.

The findings in this study do not support the concept of routine cardiotocographic screening in late pregnancy, because no case of critical fetal reserve was found in the 239 clinically normal control patients.

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Fetal acidosis, 2-chloroprocaine, and epidural anesthesia for cesarean section

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Amide-linked local anesthetic agents, such as lidocaine and bupivacaine, can become "trapped" in their ionized forms on the fetal side of the placenta, and therefore their net transfer across the placenta is increased. An ester-linked local anesthetic agent, 2-chloroprocaine, is rapidly metabolized, and placental transfer is limited. Since the metabolism of 2-chloroprocaine by fetal plasma is slower than in maternal plasma, the potential for ion trapping exists. The purpose of this study was to determine the disposition of 2-chloroprocaine and its metabolite, chloroaminobenzoic acid, in relation to the umbilical cord vein pH at delivery, and specifically, to determine whether ion trapping exists. Epidural anesthesia with 2-chloroprocaine was administered to 44 women at term prior to cesarean section. At delivery the levels of 2-chloroprocaine and chloroaminobenzoic acid in maternal plasma and umbilical cord vein were quantitated. Neonates were divided into two groups based on the umbilical cord vein pH at delivery. Ten of the 44 patients delivered neonates with a pH ≤ 7.25 (acidotic group) while 34 delivered neonates with a pH >7.25 (nonacidotic group). The mean umbilical cord vein pH in the acidotic and nonacidotic groups was 7.22 ± 0.03 and 7.32 ± 0.03, respectively ($p < 0.001$). There were also no differences between the groups in maternal and neonatal clinical characteristics or in the total dose of 2-chloroprocaine administered or the drug-to-delivery interval. The pharmacologic results did not demonstrate a statistical difference in the concentration of 2-chloroprocaine or chloroaminobenzoic acid in the maternal vein or umbilical cord vein at delivery between the two groups. Thus the results of this study demonstrate that placental transfer of 2-chloroprocaine is not influenced by fetal acidosis. Therefore these data suggest that 2-chloroprocaine may be the drug of choice when fetal acidosis or distress is anticipated. (AM J OBSTET GYNECOL 1985;151:322-4.)

Key words: Local anesthetic, fetal acidosis, chloroprocaine

The disposition of local anesthetic agents may be influenced by fetal acidosis.[1] Acidosis may cause weak bases to become "trapped" in their ionized forms on the fetal side of the placenta and increase their net transfer across the placenta. For example, elevated fetal and neonatal levels of anesthetic agents, such as lidocaine and mepivacaine, have been reported when the umbilical cord vein pH has been acidotic.[2,3] In acidotic infants, bupivacaine has also been shown to have a prolonged neonatal half-life as well as increased placental transfer.[4] Lidocaine, mepivacaine, and bupivacaine are all amide-linked local anesthetics with long plasma half-lives.

It is not clear whether ion trapping of ester-linked local anesthetic agents would also occur, since they are metabolized so rapidly. Despite its rapid metabolism, 2-chloroprocaine has been found to cross the placenta in small amounts.[5,6] Since the half-life of 2-chloroprocaine is slightly longer in fetal plasma than in maternal plasma,[7] the potential for ion trapping exists. The purpose of this study was to determine the disposition of 2-chloroprocaine and its metabolite, chloroaminobenzoic acid, in relation to the umbilical cord vein pH at delivery, and specifically, to determine whether ion trapping occurs.

Material and methods

Patients. From the obstetric population at Cleveland Metropolitan General Hospital 44 women at term were studied. Patients were scheduled for elective or repeat cesarean section. Appropriate informed consent was obtained prior to the study. Epidural anesthesia was administered by an anesthesiologist. No patients had a history of liver or kidney disease or known drug abuse.

Procedure. After identifying the epidural space by the loss-of-resistance technique, an epidural catheter was used to administer 2% or 3% 2-chloroprocaine (without epinephrine). The 2-chloroprocaine was injected as needed, and the amount injected was deter-

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Table I. Maternal, neonatal and intrapartum characteristics of the two study groups

	Acidotic group (n = 10)	*Nonacidotic group (n = 34)*
Mothers		
Age	26 ± 6*	24 ± 5
Height (in)	61 ± 1	63 ± 3
Weight (kg)	72 ± 14	80 ± 16
Hematocrit (%)	37 ± 4	35 ± 3
Neonates		
Gestational age (wk)	39 ± 1	39 ± 1
Birthweight (gm)	3362 ± 410	3395 ± 473
Apgar scores <7		
1-minute	0	0
5-minute	0	0
Umbilical cord vein pH	7.22 ± 0.03 (7.15 − 7.25)‡	7.32 ± 0.03† (7.26 − 7.41)‡
Intrapartum anesthesia		
Total dose (mg)	855 ± 326	838 ± 242
Drug-to-delivery interval (min)	38 ± 18	43 ± 21

*Mean ± SD.
†p < 0.001
‡Range.

Table II. 2-Chloroprocaine (ng/ml) and chloroaminobenzoic acid concentration (μg/ml) at delivery in maternal plasma and umbilical vein in the acidotic and normal groups

	2-Chloroprocaine (ng/ml)	*Chloroaminobenzoic acid (μg/ml)*
Maternal plasma		
Acidotic	3.0 ± 3.7	5.3 ± 4.1
Nonacidotic	3.9 ± 5.1	10.5 ± 13.1
Umbilical vein		
Acidotic	1.9 ± 3.9	3.7 ± 1.9
Nonacidotic	3.0 ± 4.1	4.9 ± 5.8
Fetal/maternal ratio*		
Acidotic	—	0.83 ± 0.3
Nonacidotic	—	0.49 ± 0.4

*Level in cord vein/level in maternal vein at delivery. Mean of individual ratios, not ratio of means.

mined by the anesthesiologist. The total dose of 2-chloroprocaine (in milligrams), the number of doses, and the time (in minutes) from administration to delivery (the drug-delivery interval) were recorded.

Sample collection. Maternal blood samples (3 ml) from a peripheral arm vein were obtained at delivery coincident with clamping of the umbilical cord. Neonatal blood samples were obtained at birth from a doubly clamped section of umbilical vein. All samples were drawn into heparinized Vacutainers containing 0.3 ml of a cholinesterase inhibitor, echothiophate iodide (0.2 gm/ml, Ayerst Laboratories), and immediately placed on ice. The plasma was removed following centrifugation and frozen until assayed for drug levels. Blood gas tension and pH were immediately measured with an automatic gas system (Corning-175).

Sample analysis. Plasma samples were analyzed separately for 2-chloroprocaine and chloroaminobenzoic acid with the use of single ion monitoring mass spectrometry[8] or electron capture gas chromatography[5] techniques, respectively. Chloroaminobenzoic acid was measured as another indicator of the placental transfer of 2-chloroprocaine.

Data analysis. Neonates were divided into two groups based on umbilical cord vein pH at delivery. Neonates with a pH ≤7.25 were considered acidotic, and those with a pH >7.25 were considered nonacidotic. The fetal/maternal ratio, i.e., the drug level in the umbilical cord vein divided by the drug level in the maternal vein at delivery, was calculated for 2-chloroprocaine and chloroaminobenzoic acid. Statistical analyses were performed with the use of the *t* statistic for two means. A p value of <0.05 was considered statistically significant.

Results

Patient population. Ten of the 44 patients in the study delivered neonates with a cord vein pH ≤7.25, while 34 patients delivered neonates with a cord vein pH >7.25. The maternal, neonatal, and intrapartum anesthetic characteristics of the two study groups are shown in Table I. All mothers were at term. There were four Class A gestational diabetics in the acidotic group and two Class A diabetics in the nonacidotic group. There were no difference in maternal age, height, weight, or hematocrit between the groups. All neonates were at term as measured by the Ballard modification of the Dubowitz exam.[9] All neonates were born with 1- and 5-minute Apgar scores of ≥7. There was no statistical difference in mean birth weights between the groups although there were six large–for–gestational age infants in the acidotic group and one large–for–gestational age infant in the nonacidotic group. Mean umbilical cord vein pH readings in the acidotic and nonacidotic groups were 7.22 ± 0.03 and 7.32 ± 0.03, respectively (p < 0.001). There were no clinical signs of 2-chloroprocaine toxicity in the neonates. There were no differences between the groups in the total dose of 2-chloroprocaine administered, the number of doses, or drug-delivery intervals.

Plasma drug levels. The maternal and cord plasma concentrations of 2-chloroprocaine and chloroaminobenzoic acid at delivery in the acidotic and nonacidotic groups are presented in Table II. There were no differences in the amount of 2-chloroprocaine (nanograms per milliliter) between the groups. The fetal maternal ratio is also shown in Table II. Since 2-chloroprocaine was often not detectable in either the mother or fetus at delivery, the fetal maternal ratio could not be calculated in the majority of patients. Chloroaminobenzoic acid was detectable in all of the maternal vein and umbilical cord vein samples collected. The fetal maternal ratio of chloroaminobenzoic acid in the acidotic and nonacidotic groups was 0.8 and 0.49, respectively. Although suggestive of increased placental transfer of 2-chloroprocaine in acidotic infants, this difference was not statistically significant.

Comment

The purpose of this study was to determine whether an ester-linked local anesthetic agent, 2-chloroprocaine, would be trapped on the fetal side of the placenta. The results of this study demonstrate that neither greater placental transfer of 2-chloroprocaine nor significantly elevated levels of chloroaminobenzoic acid occur in mildly acidotic conditions.

The fetal levels of 2-chloroprocaine were not influenced by mild fetal acidosis, as defined by an umbilical cord vein pH ≤7.25. However, findings of measurable levels of 2-chloroprocaine support previous investigations from our laboratory that demonstrate placental transfer of 2-chloroprocaine.[5,6] Furthermore, this study adds the datum that the amount of unchanged 2-chloroprocaine crossing the placenta is not significantly influenced by mild fetal acidosis.

This finding is different from that in studies of amide-linked local anesthetic agents which demonstrate greater placental transfer with fetal acidosis.[2-4] This difference between the ester and amide-linked local anesthetic agents relates to differences in their metabolism; the 2-chloroprocaine is rapidly hydrolyzed by both maternal and fetal plasma cholinesterases. The maternal and neonatal in vitro[7] half-lives of 2-chloroprocaine have been reported to be 21 and 43 seconds, respectively. The amide-linked local anesthetic agents are metabolized in the liver, with maternal and neonatal half-lives ranging from 2 to ≥8 hours depending on the drug.[10] Since all local anesthetic agents are weak bases, acidosis may cause these agents to be trapped in the ionized form. Although in theory this may occur with 2-chloroprocaine, sophisticated analytical techniques[5, 8] were unable to detect elevated levels of 2-chloroprocaine or evidence of significant increases in its metabolite in mildly acidotic conditions.[8]

The results of this study have clinical implications, since elevated or toxic levels of local anesthetic agents may be detrimental to the fetus and neonate.[11-13] Fetal acidosis or fetal distress often occur during labor at the same time that local anesthetic agents are necessary. Therefore, the results of this study suggest that 2-chloroprocaine is the drug of choice in these situations because a low or negligible level of the parent compound and an inactive metabolite may be advantageous to an acidotic or distressed fetus.

In summary, the placental transfer of the ester-linked local anesthetic agent 2-chloroprocaine is not influenced by fetal acidosis. As a result, 2-chloroprocaine may be the drug of choice for epidural anesthesia when fetal acidosis is anticipated. Furthermore, rapid placental transfer of local anesthetic agents can also follow paracervical block and local perineal infiltration.[6, 14] Therefore 2-chloroprocaine may also be the local anesthetic of choice for these types of obstetric anesthesia when fetal acidosis is anticipated.

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Collagen sponge as vaginal contraceptive barrier: Critical summary of seven years of research

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Extensive testing of collagen sponge as a vaginal contraceptive (mechanical and chemical) showed that the original expectations regarding the safety, convenience, and efficacy were not met. The collagen sponge was tested both as a cylinder and as a diaphragm and used as such or impregnated with spermicidal detergent or with zinc salt.The collagen sponge must be larger that 6 cm in diameter in order to serve as a mechanical barrier that will not be dislodged during physical activity. This creates problems with the ease of insertion and with the partners' awareness of the barrier. When the collagen sponge containing ejaculate is left in the vagina >48 hours, it develops an offensive odor. The original acidity of the collagen sponge (pH 3.5, 0.1 mol/L) is soon neutralized by the large volume of alkaline vaginal secretions. In vitro studies showed that up to 10 mg of nonoxynol 9 per milliliter of growth medium did not inhibit the growth of *Staphylococcus aureus.* These effects, as well as the large surface area of the resilient sponge, present a potential risk for growing staphylococci within the collagen sponge. The capacity of the collagen sponge to absorb a large volume of cervical and vaginal fluid produced two symptoms that were annoying to the volunteers: an awareness of either vaginal dryness during intercourse or, conversely, saturation of the sponge from the vagina. Postcoital studies showed viable spermatozoa in the cervical mucus in 25% of the tests with the nonmedicated cylindrical sponge but in only 6% of tests with the sponge containing nonoxynol 9. The results of clinical trials conducted at four centers support the view that collagen sponge as a vaginal contraceptive barrier method is inconvenient to both partners, not effective enough to compete with present methods of vaginal contraception, and possibly might be unsafe because of the capacity to grow bacteria. Despite the negative end result of this goal-oriented research, we believe that our studies have contributed to a better understanding of vaginal physiologic features, the safety and effectiveness of spermicidal detergents, and the mechanisms of vaginal malodor. Although the acceptability study showed some advantages of the collagen sponge over the rubber diaphragm, the overall acceptability of the collagen sponge diaphragm was no better than that of the rubber diaphragm. For all these reasons, including the possible risk of an increased incidence of toxic shock syndrome, we have discontinued further testing of either type of collagen sponge as a vaginal barrier method. (AM J OBSTET GYNECOL 1985;151:325-9.)

Key words: Collagen sponge, vaginal contraceptive, staphylococci, spermicide

The concept of placing a sponge in the vagina for contraception is more than 2000 years old.[1] For the past 7 years we have participated in a program in the hope of developing a sponge made of natural material which would be competitive in efficacy, safety, and convenience with other modern methods of vaginal contraception. In 1976, we presented a theoretical basis for a highly resilient and fluid-absorbent collagen sponge, having a pH of 3.5 buffered with 0.1 mol/L of citrate, to be inserted for several days in the upper vault of the vagina and thus to serve as a mechanical and chemical contraceptive barrier. It was also suggested that the high fluid-binding capacity of the collagen sponge contributed to the trapping and immobilization of the sperm within the sponge matrix.[2]

The material (collagen) and the properties of the sponge, resilience, fluid-binding capacity, inherent acidity and easy medication administration, were considered to offer new potentials. It is the aim of this report to review several years of testing on the safety, acceptability, and functional performance of the collagen sponge in the form of a cup as well as a sponge bilayer diaphragm.

Importance of the size of the insert

The dimensions of the vaginal canal depend primarily on body weight and parity of the women. For the sponge to be an effective mechanical barrier it was necessary to form a bulky and resilient structure having either an outer fitting ring or a diameter of at least 6 cm.

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Table I. Incidence of dripping with collagen sponge

Time of retention (hr)	*Incidence of dripping (%)*
2	9
6	36
12	81
24	82
48	82

The study involved 11 volunteers, who tested a total of 66 sponges with or without nonoxynol 9.

We tested the collagen sponge for proper placement and retention. The diameter of the sponges varied from 5 to 8 cm, and the width was always 2.5 cm. We found a high incidence of dislodgement of the sponge that was 5 cm in diameter. The sponges that were 6 centimeters in diameter were acceptable for nulliparous women. Parous or obese women would need sponges of 7 to 8 cm in diameter. The latter size was difficult to insert, either digitally or with an applicator (see below). We concluded that the sponge should be at least 6 cm in diameter by 2.5 cm long to be retained in the upper vault of the vagina.[3] We also found that sponges <2.5 cm thick often folded in the fornix.

Insertion of collagen sponge

Although originally we recommended digital insertion of a moist and compressed sponge, testing indicated that application with an inserter resulted in more reproducible and appropriate placement of the collagen sponge adjacent to the cervix. The longer applicator produced the best placement of the sponge.[3, 4]

Removal of collagen sponge

As the collagen sponge needs to be rather voluminous to function as a mechanical barrier, it is difficult to remove from the upper vault of the vagina. We tested various methods of removal and found that a loop made of polyfilamentous Dacron fibers facilitated the process.[3, 4]

Time of retention of collagen sponge in the vagina

Sexually inactive volunteers were able to keep the sponge in place for 2 to 28 days without developing a malodor. However, in sexually active volunteers the offensive odor developed in most users after 2 days of vaginal retention of the sponge.[5] In several studies we analyzed the chemical basis of vaginal malodor formation [6, 7] and also studied the factors controlling it.[8] The major contributor to vaginal malodor is the ejaculate, high in polyamines such as spermine and spermidine. The effect of aminooxidases, functioning at neutral pH and in the presence of copper, is to metabolize the polyamines to propylamine and butylamine which are responsible for the offensive odor. Acidity and zinc ions inhibit the enzyme(s) involved in the formation of the malodorous products. However, soon after intravaginal insertion, the initial acidic pH of the sponge is neutralized by the vaginal secretion. Also, volunteers using zinc-medicated sponges complained of vaginal dryness. Thus, both potential methods of controlling the formation of vaginal malodor were found by in vivo testing to be either ineffective or impractical.

Table II. Volume of cervical-vaginal fluid recovered in five volunteers in 12 hours

Type of sponge tampon	*Secretion/12 hours (gm)*
Plain sponge	9.7 ± 2.1
Sponge with nonoxynol 9	10.3 ± 2.5

Data presented as mean ± SD are based on at least five observations per woman during days 12 to 16 of the menstrual cycle.

Vaginal secretion

One of the unpleasant side effects of vaginal contraception is the uncontrollable loss of fluid from the vagina (dripping). The collagen sponge retained in the vagina for a period >12 hours was soaked with 20 to 40 ml of fluid. Approximately 5% of volunteers who tested the glutaraldehyde–cross-linked collagen sponge noticed excessive dripping. This may be a hypersensitivity phenomenon analogous to contact dermatitis caused by glutaraldehyde.[9]

We studied the volume of vaginal secretion and related this to the hours of vaginal sponge retention. Eleven volunteers used a total of 66 collagen sponges with and without nonoxynol 9 (100 mg per sponge), inserted into the vagina for 2 to 48 hours (Table I). Most volunteers reported marked dripping if the collagen sponge was inserted for >12 hours. There was no difference in the incidence of dripping between sponges with and those without the spermicidal detergent (100 mg of nonoxynol 9 per sponge). Sexual activity did not increase the incidence of dripping.[10]

In another study involving five volunteers the actual volume of vaginal secretion absorbed by the inserted collagen sponge cylinders (10 cm long, 2.5 cm wide) was measured. Preweighed sponges with or without 100 mg of nonoxynol 9 spermicidal detergent were left in the vagina for 12 hours by women on days 12 to 16 of the menstrual cycle. Recovered vaginal and/or cervical fluid amounted to 20 ml/24 hours (Table II). The spermicidal detergent did not affect the volume of the collected fluid.[10]

Table III. Effect of various concentrations of glutaraldehyde on the growth of *S. aureus*

Glutaraldehyde in medium (mg/ml)	S. aureus *growth (in corrected turbidity units)*
0	1.2
0.62	1.2
1.25	0.45
4.17	0
12.5	0

Growth measured turbidimetrically at 675 nm.

Antibacterial properties of contraceptive collagen sponges

Because of the risk of toxic shock syndrome it became essential to develop a sponge that would not favor the growth of staphylococci. We recognized that the collagen sponge with the complexity of its surface morphologic features and richness of continuous opened channels would be an excellent matrix for bacterial growth.

pH. We postulated that making the collagen matrix acidic with a pH of 3.5 and buffering the acidity by 0.1 mol/L of citrate buffer should prevent any bacterial growth. The growth of a coagulase-positive strain of *Staphylococcus aureus* at various pH levels of the culture medium, consisting of trypticase soy broth with 1.0% added yeast extract (GIBCO, Chagrin Falls, Ohio), was investigated. The results (Fig. 1) indicate that moderate inhibition of *S. aureus* growth occurred at pH 5.5; the growth was measured turbidimetrically at 675 nm. There was no measurable growth at ≤pH 4.2. Thus, maintaining the vaginal sponge pH within the range of pH 3.5 to 4.0 might be a simple and innocuous method of preventing the possible growth of *S. aureus*. Direct determination of the pH of the sponges recovered after 2 hours of vaginal wear and extracted with distilled water showed no change of the original pH (3.5). However, at 6 hours the pH was 4.5 ± 0.4, and at 12 hours and later the pH of the sponge was 7.2 ± 0.6. An increase of the buffering capacity of the citrate buffer above a strength of 0.2 mol/L resulted in irritation of the vagina (itching). It is obvious that a slightly alkaline vaginal secretion with high buffering capacity neutralized the pH of the sponge within a few hours.

Glutaraldehyde. Collagen sponges for use as vaginal barriers were cross-linked with glutaraldehyde. This tanning agent cross-links the sponge and renders it resilient and insoluble in biologic fluids. It is also retained within the framework of the sponge in the form of a polymer, which is continuously hydrolyzed, especially at an acid pH, and leaks out of the sponge.[11, 12] Glutaraldehyde is known for its bactericidal effect,[13] and we speculated that the content of "free, leakable"

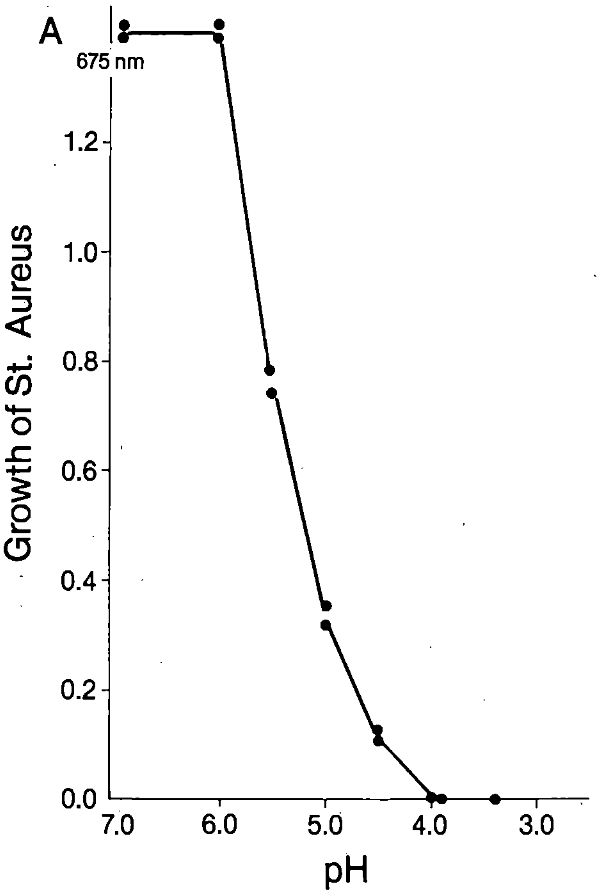

Fig. 1. The effect of the pH of the incubation medium on the growth of *S. aureus*. A coagulase-positive stain (ATCC No. 25923) of *S. aureus* was incubated for 24 hours in trypticase soy broth with 1% added yeast extract, adjusted to pH values shown by phosphate or citrate buffers. The growth was measured turbidimetrically at 657 nm.

glutaraldehyde would exert its bactericidal effect within the sponge. Tests were performed to determine the effect of different concentrations of glutaraldehyde in the culture medium on the growth of *S. aureus* (ATCC No. 25923).

The results (Table III) showed that glutaraldehyde at a concentration of 1.25 mg/ml in the medium partially inhibits the growth of this strain of *S. aureus*. The growth of *S. aureus* was completely inhibited at 4.2 mg of glutaraldehyde per milliliter.

Although the mechanism of the effect of glutaraldehyde is not clear, the results are of academic value, as the maximum amount of free glutaraldehyde released during the extraction of a single contraceptive collagen sponge for 24 hours in acetate buffer, pH 3.5, was of one magnitude below the effective bacterial growth–inhibition dose.

Nonoxynol 9. Finally, we tested the possible bactericidal effect of the spermicidal detergent nonoxynol

Table IV. Release of spermicidal detergent Igepal Co-630 into the vagina of volunteers using the sponge diaphragm for varying intervals

Duration of wear (hr)	*Retention of detergent (mg/sponge)*
0	93.0 ± 6.1
	$p > 0.05$
2	55.3 ± 6.7
6	37.9 ± 7.5
	$p < 0.05$
6 (with intercourse)	23.3 ± 4.1
12	14.2 ± 3.2
24	22.2 ± 4.1
48	19.9 ± 4.5

Sponges containing 93.0 ± 6.1 mg of Igepal Co-630 were retained in the vagina for 2 to 48 hours. In the extract of recovered sponges the detergent was determined by high-performance liquid chromatography with a fluorometric detector by the procedure of C. Eskelson (unpublished data).

Variability is given as mean ± SD.

Table V. Effectiveness of various treatments of cylindrical collagen sponge on the postcoital test

Treatment	*Positive tests (in % of total)*
Collagen sponge, untreated	22
Collagen sponge with 40 mg zinc sulfate and $7H_2O$	20
Collagen sponge with 100 mg nonoxynol 9	6*
Collagen sponge with spermicidal cream	14

*Significantly lower ($p < 0.05$) as compared with nonmedicated collagen sponge.

9. It has been shown that certain surface-active agents alter the interface between the lipid-containing membrane of a bacterial cell and the surrounding medium in such a way that the membrane becomes structurally labilized.

The same strain of *S. aureus* was incubated for 24 hours at various concentrations of nonoxynol 9 (Igepal Co-630, GAF). We found that within the range of 0.05 to 10.0 mg of nonoxynol 9 per milliliter of medium, nonoxynol 9 did not inhibit the growth of *S. aureus.* This result is in agreement with the general view that nonionic detergents are relatively harmless to the majority of bacterial cells.[14]

Collagen sponge with zinc ions

The inhibitory effects of zinc ions on various functions of spermatozoa have been well documented.[15] We showed that zinc sulfate added to sponges (40 mg of zinc sulfate and $7H_2O$ per sponge) does not irritate the vaginal mucosa of the rabbit.[16] When zinc-containing sponges were used by volunteers, we did not detect any increase in the serum content of zinc. A common complaint was dryness of the vagina in sexually active volunteers. Because of this effect we abandoned the use of zinc in intravaginal applications for contraceptive purposes.

Collagen sponges with nonoxynol 9 spermicidal detergent

It has always been our goal to develop a sponge that would serve as both a mechanical and a chemical contraceptive barrier.[2, 17] Originally we believed that the acidity of the sponge would suffice to inactivate the absorbed spermatozoa. Realizing the temporary nature of the acid environment of the sponge, we added a detergent, nonoxynol 9, to the sponge.

The use of nonoxynol 9 was subjected to several studies, involving its pharmacokinetics,[18] its tolerance in the vagina of rabbits,[19] its toxic effect against fibroblasts as compared with spermatozoa,[20] and finally its effect on the morphologic features and biochemistry of the liver and kidney.[21] In these studies we learned that 85% of vaginal nonoxynol 9 is recovered in the urine and feces. The rate of absorption of nonoxynol 9 by the vaginal wall depends on the vehicle. Fibroblasts in tissue culture were found to be more sensitive than spermatozoa to the effect of nonoxynol 9. Finally, we found evidence of hepatotoxicity and nephrotoxicity in rats and rabbits given high dosages of nonoxynol 9, administered into either the peritoneal cavity or the vagina.

In a clinical study we tested the rate of release of nonoxynol 9 from the sponge in women (Table IV). It has to be stressed that nonoxynol 9, as an aqueous solution, was added to the sponge which was then dried. Surprisingly, we found that after 2 hours of wear, almost 50% of the nonoxynol 9 was lost from the sponge. Intercourse significantly increased the release of nonoxynol 9 from sponge. Only about 20% of the original amount of nonoxynol 9 was left in the sponge worn for 24 and 48 hours in sexually inactive women.

Postcoital tests with collagen sponge

The effectiveness of the collagen sponge alone, medicated with zinc or containing a spermicidal detergent to prevent the propagation of spermatozoa into the cervical mucus, was studied in a total of 103 postcoital tests. A comparison study of the sponge with a commercial spermicidal cream was also made.[22] The results (Table V) showed that the highest failure rates of 22% and 20% were for the sponge alone or the one containing zinc. The presence of the spermicidal detergent reduced the incidence of positive tests to 6%.

Clinical testing of contraceptive effectiveness

Testing of a collagen sponge cup was conducted at several centers. The initial pilot study was performed at Texas Tech University, Lubbock, Texas, under the direction of Dr. Heine. There were 57 women in the study. A large dropout rate was due to patients moving out of the area and because of patients' and their partners' complaints. There were a total of 267 woman months, 191 woman months with the sponge containing 100 mg of nonoxynol 9 and 76 woman months with the nonmedicated sponge. There were eight pregnancies, resulting in a Pearl index of 35.9 for 100 women.

In another clinical trial, conducted in North Carolina by Dr. G. S. Berger, the net cumulative pregnancy rate at 6 months was 20.3 per 100 women. Based on a total of 130 woman months of exposure, the Pearl index was 55.4 per 100 women. The overall experience in two other testing centers (the Noi Klinika in Debrecen, Hungary, and the Margaret Pyke Center, London, England) was similar.

In conclusion, thorough analysis of the above-reported results as well as a possible risk of an increased incidence of toxic shock syndrome led us to discontinue further testing of either type of collagen sponge as a vaginal barrier method.

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Autonomic hyperreflexia: A mortal danger for spinal cord–damaged women in labor

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Reproductive care of women with spinal cord damage demands knowledge of such women's reproductive potential and the specific complications to which these women are prone during pregnancy and childbirth, especially autonomic hyperreflexia. Fertility in cord-damaged women of reproductive age is generally undiminished as are libido, ability to have intercourse, and ability to bear children. Frequent complications of cord-damaged pregnant women include urinary tract infection, anemia, pressure sores, sepsis, unattended birth, and autonomic hyperreflexia. Autonomic hyperreflexia or autonomic dysreflexia occurs during labor in up to two thirds of women with cord lesions above T-6. Autonomic hyperreflexia results from noxious stimuli including distention of the bladder, cervix, or rectum, which evokes mass triggering of sympathetic and parasympathetic afferents that are uninhibited by supraspinal centers below the cord lesion. Autonomic hyperreflexia manifests itself with sudden onset of marked hypertension and headache during uterine contractions, as well as bradycardia or tachycardia, various cardiac dysrhythmias, and marked diaphoresis with piloerection and flushing above the level of the cord lesion. We describe the second reported occurrence of intraventricular hemorrhage due to autonomic hyperreflexia during labor and detail recommendations for anticipating and mitigating this potentially lethal complication of parturition in cord-damaged women. Pregnancy and parturition are best carried out with informed cooperation of the patient and of obstetric, cord rehabilitation, anesthetic, and nursing personnel. (AM J OBSTET GYNECOL 1985;151:330-3.)

Key words: Autonomic hyperreflexia, spinal cord damage, labor

Improvement of acute and rehabilitative care allows for an ever-increasing number of cord-damaged women not only to survive but to lead increasingly independent lives, including sexual expression, reproduction, and founding or continuation of families. In the past, medical care providers considered paraplegia or quadriplegia as contraindications to pregnancy. More recently, it has been recognized that after medical stabilization and pyschological readjustment from cord damage, female libido, as well as reproductive capability, remains intact.[1-7] Multiple observations in humans and animals demonstrate that sexual appetite returns and that sexual performance, although physically impeded, may be satisfying for both partners.[2, 5, 8] Rehabilitation authorities recommend both continuation and founding of families as a further means of completing readjustment and return to more independent life.[1-8] Spinal cord injury is frequently associated with transient anovulation but the preinjury menstrual pattern is reestablished in a matter of months. Unlike paraplegic men, women with cord damage appear to be fully fertile and do not appear at excess risk of spontaneous abortion or birth defects.[2, 5] Appropriate reproductive care for cord-damaged women includes both sexual and reproductive counseling, including provisions for contraception and pregnancy.

The highly variable but potentially lethal syndrome of autonomic hyperreflexia occurs in up to two thirds of cord-damaged laboring women whose lesions are above the T-6 level.[9] Since few care providers are familiar with the particular problems of pregnancy and delivery in cord-damaged women, we describe the second reported occurrence of cerebral hemorrhage due to autonomic hyperreflexia in an otherwise healthy paraplegic woman during labor so as to facilitate care of similar women.

Case report

This 30-year-old, white woman, gravida 1, para 1, became a C6-7 quadriplegic during an automobile accident in 1971. The patient became rehabilitated to an independent living status with the assistance of her husband and became pregnant. Her prenatal course was uncomplicated except for recurrent urinary tract infection associated with an indwelling suprapubic catheter. At 39 weeks' gestation, labor was electively induced by intravenous oxytocin infusion and amniotomy so as

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to avoid an unattended birth. After 2 hours of induced labor, the cervix was found to be dilated to 8 to 9 cm with the vertex at the 0 station. The patient was noted to be perspiring perfusely over the face and neck. Concurrently, she developed the sudden onset of severe posterior neck and occipital headache. Blood pressure was measured at 210/100 torr and she was given 5 mg of hydralazine intravenously. Within minutes the cervix was noted to be completely dilated and the vertex at a +1 station. Blood pressures were 150/90 and 170/100 torr. Ten minutes after entering the second stage of labor, the patient became incoherent and nonresponsive. A 3118 gm male infant with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively, was delivered by low forceps. The infant's subsequent course and development were normal. The mother remained comatose with unequal and nonreactive pupils. She was responsive only to deep pain and became apneic with labile blood pressure in the hours after birth. A computed tomographic scan revealed massive intraventricular hemorrhage in the lateral and third and fourth ventricles. Therapy included ventriculostomy and tracheal intubation with subsequent tracheostomy and administration of corticosteroids. Carotid artery arteriograms on the second postpartum day revealed diffuse vascular spasm of the proximal intracranial vessels without revealing any bleeding site. After a stormy 2-week course of supportive care, the patient began to trigger the respirator spontaneously. On the twenty-eighth postpartum day, she was extubated and 2 days later was able to respond to verbal commands. Over the ensuing weeks, the patient slowly improved to her present ability to comprehend and speak. She regained her previous partial arm and hand function but retains a right hemianopia and extraocular muscle dysfunction not previously noted along with defects of short-term memory and cognitive function.

Comment

The syndrome of autonomic hyperreflexia or dysreflexia in patients with cord damage was first reported by Head and Riddoch[10] in 1916 and subsequently by others.[11-14] Autonomic hyperreflexia is now well recognized as a feature of disordered neurological response to noxious stimuli occurring below the level of cord damage, characteristically above the seventh to fifth thoracic vertebrae. Autonomic hyperflexion begins in the months after recovery from spinal shock.[13] Noisome visceral stimulation such as bladder, rectal, or cervical distention, contraction, or manipulation ascends in spinothalamic tracts and posterior columns.[13] Other painful stimuli that elicit autonomic hyperreflexia include strong suprapubic bladder pressure, perineal manipulation during disimpaction, excessive deep breathing, and immersion of feet in cold water.[13] Such stimuli elicit a spinal reflex of sympathetic and parasympathetic outflow which is uninhibited by either supraspinal centers below the lesion or elsewhere in the central nervous system.[13] Acute hypertension is mediated by peripheral and splanchnic vasoconstriction and commonly reaches levels of 200 torr systolic and 150 torr diastolic.[13] Intense vagal stimulation results from stimulation of aortic arch and carotid sinus receptors and mediates bradycardia and cardiac arrhythmias.[13] Marked diaphoresis and piloerection limited to dermatomes above the cord lesion result from undamped increased autonomic tone with release of catecholamines and/or prostaglandins into the circulation.[13, 15, 16] Most investigators feel that the characteristic sudden onset of hedache and possible encephalopathy and cerebrovascular accident is due directly to the sharply increased blood pressure which occurs in synchrony with the noxious stimuli.[13, 15] Marked flushing above the level of the lesion along with unilateral pupil dilation and nasal stuffiness is also frequently noted. Specific effects of increased levels of various catecholamines and prostaglandins on uterine activity remain unstudied, although labor is anecdotally rapid.[16]

Hypertensive crisis with possible encephalopathy and cerebrovascular accident is well described in nonpregnant cord-damaged patients.[13] However, only one other incidence of cerebrovascular accident due to autonomic hyperreflexia has been adequately described during pregnancy. Abouleish[15] reported a maternal death from intracranial hemorrhage due to autonomic hyperreflexia which was mistaken for preeclampsia. Since as many as two thirds of carefully observed pregnant women with cord lesions at T-6 or above develop autonomic hyperreflexia to some degree during labor, it is likely that similar episodes of cerebrovascular accident have been misdiagnosed as due to pregnancy-associated hypertension.[9]

Avoidance and expeditious handling of possible autonomic hyperreflexia in labor remain the overriding concern in the care of cord-damaged women in labor. The occurrence of autonomic hyperreflexia appears limited to women with cord transection above T-6. Partially expressed syndromes occur with incomplete cord severance.[4, 9, 17] As with all cord-damaged individuals, noxious stimuli should be avoided at all times in patients with a history of autonomic hyperreflexia. Vaginal examination, Foley catheter placement, or rectal manipulation should be preceded by topical application of anesthetic jelly to reduce stimulation. Mandatory bladder catheter drainage during labor should be repeatedly monitored so as to avoid obstruction with bladder distention and subsequent autonomic hyperreflexia.[20] Unlike the more common hypertensive complications of pregnancy, such as preeclampsia-eclampsia, the elevated blood pressure associated with autonomic hyperreflexia builds rapidly with the contraction and abates markedly between uterine contractions and the entire constellation of changes disappears rapidly after delivery.[1] Blood pressure determinations between uterine contractions do not accurately reflect the condition or response to antihypertensive therapy.

Watson and Downey[18] suggested that regional anesthesia, such as epidural or spinal block, is optimal for preventing and controlling autonomic hyperreflexia during labor. Regional anesthesia has been used to eliminate autonomic hyperreflexia in cord-damaged nonpregnant individuals with success.[19] Fears regarding use of regional anesthesia in patients with neurological damage may have to be overcome with consultation well before the onset of labor. Use of paracervical block to eliminate neurological stimulation during cervical dilatation remains unstudied but should be accompanied with direct fetal monitoring if attempted. Since abdominal pressure may initiate autonomic hyperreflexia, fetal monitoring is likely best performed either with periodic auscultation by Doppler devices or by continuous fetal scalp electrode monitoring with avoidance of constraining external abdominal belts.[20] Frequent maternal monitoring with observation for signs of autonomic hyperreflexia, such as perspiration, flushing, and elevated blood pressure during contractions, is mandatory. Blood pressure monitoring should be frequent, if not continuous, when the patient is in active labor. Robertson[17] and Tabsch et al.[20] recommend placement of an arterial line to continuously measure blood pressure, but frequent cuff monitoring appears satisfactory.

If autonomic hyperreflexia is not prevented or satisfactorily mitigated by a regional anesthetic, then short-acting antihypertensive agents should be given under careful control. There is little information on agents of choice in this situation in which both the maternal and the fetal condition must be closely monitored. While medications such as hydralazine or diazoxide have been more commonly used in pregnancy, there is considerable experience with pump- or drip-infused solutions of ganglionic blockers such as trimethaphan, which have been successfully used to control autonomic hyperreflexia in nonpregnant patients.[21-24] Use of any of these agents or nitroprusside infusion should be accompanied with close monitoring of effective intravascular volume, preferably with central venous pressure line readings to guide fluid management.[20, 24] With evidence of continuing autonomic hyperreflexia during the second stage of labor, labor may be reasonably shortened with forceps- or vacuum extraction–assisted delivery with adequate anesthesia. Even though delivery by cesarean section is associated with prompt cessation of autonomic hyperreflexia, cesarean section should be reserved for obstetric indications.[6, 8, 17] In any of these circumstances, optimal care depends on the cooperation between obstetric, anesthetic, rehabilitative, and nursing personnel and the patient herself and must be initiated long before the crisis of autonomic hyperreflexia supervenes.

In summary, reproductive care of spinal cord–damaged women demands knowledge of these individuals' reproductive potential and the specific complications which put these women at risk during pregnancy and delivery. Of these, autonomic hyperreflexia, a syndrome resulting from undamped autonomic stimulation, occurs in as many as two thirds of women with cord lesions above T-6. Autonomic hyperreflexia manifests itself with flushing and piloerection above the cord lesion level and acute hypertension which may lead to hypertensive crisis and cerebrovascular hemorrhage and possible death. Prevention and treatment of autonomic hyperreflexia require preplanning and coordinated care of cord-damaged women in labor by obstetric, anesthetic, and nursing care providers.

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Estimation of fetal weight with the use of head, body, and femur measurements—A prospective study

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In utero estimates of fetal weight were evaluated prospectively in 109 fetuses with the use of sonographic models developed in a previous study. This report confirms that the best in utero weight estimates result from the use of models based on measurements of head size, abdominal size, and femur length. Since the accuracy of these models (1 SD = 7.5%) is significantly better than those based on measurements of head and body (e.g., biparietal diameter, abdominal circumference), we recommend routine use of such models in obstetric sonography. (AM J OBSTET GYNECOL 1985;151:333-7.)

Key words: Fetal weight, ultrasound

In a previous report[1] we demonstrated that the most accurate estimates of fetal weight in utero are those based on at least three fetal measurements—biparietal diameter or head circumference as an index of head size, abdominal circumference as an index of body girth, and femur length as an index of crown-heel length. The purpose of the current study was to evaluate this method of estimating fetal weight prospectively in a new fetal population.

Material and methods

The study population consisted of 109 predominately middle class Caucasian patients examined by physicians by means of commercially available linear-array real-time systems (ADR models 2130 and 4000, Siemen's model 2380, and Technicare model 256). The majority of patients were examined within 3 days of delivery, and all patients were examined within at least 1 week of delivery. The study population was unselected and included preterm, term, and postterm fetuses as well as fetuses that were growth retarded or macrosomic. The imaging and measurement techniques used have been previously described in detail.[1]

The fetal weight was estimated in this population by means of models from our previous study.[1] These estimates were compared with weight estimates with the use of the model of Shepherd et al.,[2] which is based on measurements of biparietal diameter and abdominal circumference. The errors in predicting fetal weight were expressed as a percentage of actual birth weight by means of the following method:

Error (%) = predicted weight − actual weight/actual weight × 100

The t test was used to determine if the mean errors were different from zero, and the F test was used to determine if there were significant differences in the standard deviations of the mean errors.

Results

The accuracy of our original models,[1] as well as the model of Shephard et al.,[2] are summarized in Table I. As noted in our previous study, the combination of abdominal circumference and femur length and all

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Table I. Summary of accuracy of models

Parameters*	Model	*Mean deviation ± SD (%)†* Total (N = 109)	<1500 gm (n = 13)	1500-2000 gm (n = 14)	2000-2500 gm (n = 15)	2500-3000 gm (n = 12)
Biparietal diameter, abdominal circumference	Shepard et al.[2]	1.3 ± 10.1	−3.9 ± 10.0	1.2 ± 10.2	3.5 ± 15.1	−0.2 ± 11.1
Head circumference, abdominal circumference	Hadlock et al.[1]	1.5 ± 9.8	−0.6 ± 10.6	−1.4 ± 12.9	4.9 ± 13.6	−2.8 ± 8.8
Abdominal circumference, femur length	Hadlock et al.[1]	0.4 ± 7.7	−3.9 ± 8.3	0.9 ± 8.5	1.6 ± 8.5	0.3 ± 7.6
Biparietal diameter, abdominal circumference, femur length	Hadlock et al.[1]	1.4 ± 7.3	−5.3 ± 9.0	2.2 ± 7.0	3.2 ± 7.6	1.3 ± 7.7
Head circumference, abdominal circumference, femur length‡	Hadlock et al[1]	2.3 ± 7.4	−4.6 ± 9.7	2.5 ± 7.4	4.9 ± 7.3	1.7 ± 6.6
Biparietal diameter, head circumference, abdominal circumference, femur length	Hadlock et al.[1]	−0.7 ± 7.3	5.4 ± 9.0	−1.4 ± 7.0	−2.6 ± 7.8	−0.4 ± 7.4

*Fetal measurements in cm; fetal weight in gm.
†Deviation(%) = predicted weight − actual weight/actual weight × 100.
‡Mean difference statistically significant ($p = 0.05$).

Table II. New regression models based on an expanded sample population (n = 276 fetuses)

Fetal parameters	*Regression equations**
Abdominal circumference, femur length	Log_{10} weight = 1.304 + 0.05281 AC + 0.1938 FL − 0.004 AC × FL
Biparietal diameter, abdominal circumference, femur length	Log_{10} weight = 1.335 − 0.0034 AC × FL + 0.0316 BPD + 0.0457 AC + 0.1623 FL
Head circumference, abdominal circumference, femur length	Log_{10} weight = 1.326 − 0.00326 AC × FL + 0.0107 HC + 0.0438 AC + 0.158 FL
Biparietal diameter, head circumference, abdominal circumference, femur length	Log_{10} weight = 1.3596 − 0.00386 AC × FL + 0.0064 HC + 0.00061 BPD × AC + 0.0424 AC + 0.174 FL

*AC, abdominal circumference; FL, femur length; BPD, biparietal diameter; HC, head circumference.

combinations of three or more parameters resulted in significantly ($p = 0.05$) better weight estimates than those using measurements of head and abdomen (e.g., biparietal diameter and abdominal circumference, head circumference and abdominal circumference). The largest random errors (the standard deviation is an index of random errors) resulted from use of the model of Shephard et al.[2]; the size of the error (1 SD = 10.1%) is identical to the standard deviation of the regression originally reported by Warsof and associates[3, 4] with use of the biparietal diameter and abdominal circumference. The standard deviation for our head circumference and abdominal circumference model is slightly higher (9.8% versus 9.1%) than previously reported.[1] The standard deviation for our abdominal circumference and femur length model is slightly lower (7.7% versus 8.2%) than previously reported. These differences, and the minor differences in standard deviations for biparietal diameter, abdominal circumference and femur length (7.3% versus 7.7%), head circumference, abdominal circumference, and femur length (7.4% versus 7.6%), and biparietal diameter, head circumference, abdominal circumference, and femur length (7.3% versus 7.5%), are not statistically significant ($p = 0.05$).

Of some concern is the finding of small systematic errors (in this context the mean deviation is an index of systematic error) for several of the models (Table I). The largest systematic error was 2.3% for the head circumference, abdominal circumference, and femur length model. The reason for this systematic error is not readily apparent; it may be related to the fact that

Mean deviation ± SD (%†)		
3000-3500 gm (n = 20)	*3500-4000 gm (n = 16)*	*>4000 gm (n = 19)*
1.5 ± 8.4	2.8 ± 8.8	2.5 ± 7.1
−0.8 ± 6.6	4.2 ± 7.5	5.1 ± 5.9
−1.9 ± 7.2	−0.7 ± 6.8	5.2 ± 5.2
0.1 ± 6.0	1.4 ± 7.1	4.8 ± 5.1
0.2 ± 5.9	3.2 ± 6.9	6.3 ± 5.1
0.9 ± 5.9	−1.2 ± 7.2	−4.0 ± 5.2

many of the examinations in this study were performed by physicians with less experience than those in the previous study.

Comment

Although the mean deviation and standard deviation of a regression model from a given population are useful indices of the magnitude of the systematic error and random error which one could expect using the model, it is important to keep in mind that there is an inherent bias in favor of the model, since it is in effect being tested on the population from which it was developed. It is appropriate therefore to initiate further testing of the model(s) in a new population of patients. The results from the 109 fetuses in this population indicate that the systematic and random errors for the models generated in our previous study are accurate estimates of these errors in our general population.

A second question that one should attempt to answer is whether the original sample population was large enough to be truly representative of a general population of fetuses. In order to answer this question, we combined the original study population (n = 167) with the current study population (n = 109) to form a composite population of 276 fetuses. We then evaluated the various combinations of fetal parameters previously reported,[1] using regression analysis to determine whether improvements in the accuracy of the weight-estimating procedure (as indicated by the standard deviation of the regression) could be achieved by the increased sample size. The differences in the accuracy of the old and new models, which are summarized in Tables II and III, are not statistically significant ($p = 0.05$). There was a slight increase in the coefficient of determination (r^2) for the models based on the population of 276 fetuses, which indicates that these models explain a slightly higher percentage of the observed variability than the previous models. We do not feel that these differences are clinically significant.

Table III. Comparison of weight-estimating models derived from fetal populations of different sizes*

Fetal parameters	*Mean deviation ± SD (%)*	*Coefficient of determination (%)*
Head circumference, abdominal circumference		
Model 1	0.4 ± 9.1	95.2
Model 2	0.4 ± 9.1	96.5
Abdominal circumference, femur length		
Model 1	0.3 ± 8.2	96.0
Model 2	0.3 ± 8.0	97.3
Biparietal diameter, abdominal circumference, femur length		
Model 1	0.3 ± 7.7	96.5
Model 2	0.3 ± 7.5	97.6
Head circumference, abdominal circumference, femur length		
Model 1	0.3 ± 7.6	96.5
Model 2	0.0 ± 7.5	97.6
Biparietal diameter, head circumference, abdominal circumference, femur length		
Model 1	0.3 ± 7.5	96.5
Model 2	0.1 ± 7.4	97.7

*Model 1 refers to our original study[1] (n = 167). Model 2 refers to a combined population (n = 276); the regression equations are listed in Table II.

One fetus that we have evaluated subsequent to this study provides a useful example of why it is important to analyze head size, trunk size, and length when attempting to estimate fetal weight in utero. This fetus, which had profound microcephaly secondary to an early viral infection in utero, had the following measurements: biparietal diameter, 5.7 cm; head circumference, 21.3 cm; abdominal circumference, 28.5 cm; femur length, 7.5 cm. The weight estimation based on the model of Shepherd et al.[2] using biparietal diameter and abdominal circumference resulted in an error of

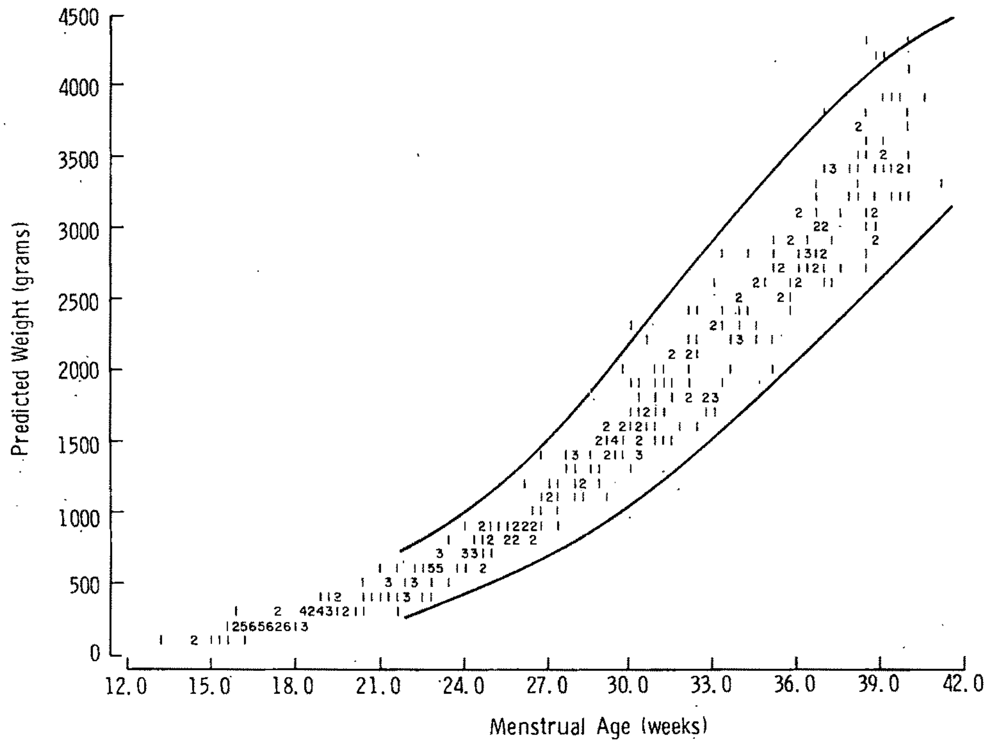

Fig. 1. Distribution of weight estimates in 361 normal pregnancies with the use of the head circumference, abdominal circumference, and femur length model.[1] The *solid lines* represent the 10th and 90th percentiles for birth weight based on the study of Williams et al.[6]

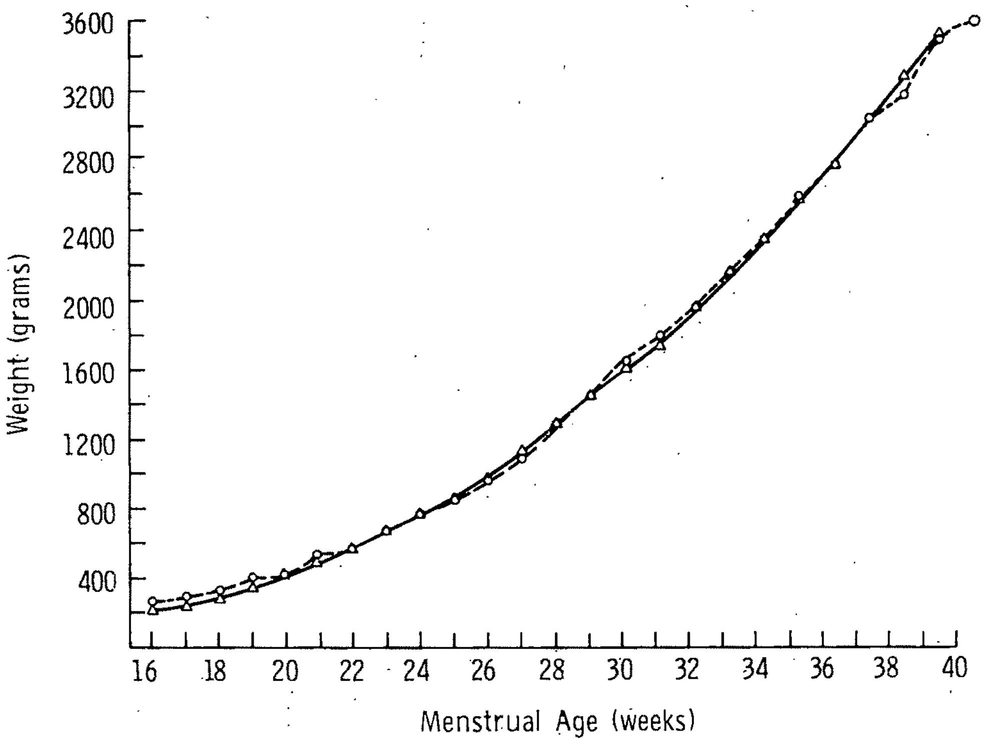

Fig. 2. Mean predicted weight at each week of gestation with use of the head circumference, abdominal circumference, and femur length model[1] in 361 normal pregnancies (o---o) compared with the expected weight at each week of gestation based on the longitudinal study of Deter et al.[7] (△——△).

1197 gm (46.8%), whereas the model using head circumference, abdominal circumference, and femur length resulted in an error of 165 gm (7.3%) when compared to the actual birth weight of 2250 gm.

A recent report[5] has questioned the validity of currently available in utero weight standards such as the study by Williams et al.[6] of over two million newborn infants. The argument against such standards is based on the premise that preterm deliveries (which are used in part to create these standards) are not normal physiologic events and that it may be erroneous to assume that these are normal fetuses. Ott and Doyle[5] has suggested the use of a new fetal weight standard based on in utero sonographic weight estimates of 186 fetuses by means of the model of Shephard et al.[1] (biparietal diameter and abdominal circumference). Given the inherent error in this weight-estimating procedure (2 SD = ±20%),[2-4] we feel that the weight standard suggested by Ott and Doyle[5] should not be substituted for standards such as those of Williams et al.[6] In fact, when we evaluated our optimal model[1] (head circumference, abdominal circumference, and femur length) on 361 normal fetuses in utero, the shape of the weight curve was identical to that reported by Williams et al.[6] (Fig. 1), and almost all data points fell within the normal range. Moreover, when we compared the mean weight estimate at weekly intervals in this population with the predicted weight based on the longitudinal study of Deter et al.,[7] the growth curves were virtually identical (Fig. 2). We conclude that weight standards such as those of Williams et al.[6] are appropriate standards of normal growth for the populations from which they were derived. When choosing such a standard for one's own population, it is of obvious importance to consider race, sex, socioeconomic factors, geographic locale, or any other factor that may influence the normal weight range in a given population of fetuses.[8]

In summary, our study establishes two points: (1) it confirms that the addition of femur length to head and abdomen measurements increases the accuracy of in utero weight estimates based on ultrasound studies, and (2) it demonstrates that the regression models from our original study[1] based on 167 fetuses are accurate estimators of weight in our general population. In our previous report we suggested that the head circumference is a better index of head size than the biparietal diameter (primarily because of its relative shape independence) and that the head circumference, abdominal circumference, and femur length model could be considered the best overall model. Because of the small systematic error observed in use of the original head circumference, abdominal circumference, and femur length model in our prospective population, we suggest that the new head circumference, abdominal circumference, and femur length equation in Table II be considered the optimal weight-estimating model for general use. Clearly, when a given measurement is technically inadequate or impossible to obtain (e.g., the head measurement when the head is deeply engaged), a model should be chosen based on the measurements available. Finally, it must be emphasized that population differences or subtle differences in imaging and measurement techniques may change the form of the optimal equation or the values of its coefficients.[1]

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Haemophilus influenzae causes purulent salpingitis

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We describe two patients with laparoscopically diagnosed, severe acute salpingitis who had nontypable *Haemophilus influenzae* isolated directly from the fallopian tubes. Nontypable *H. influenzae* should be recognized as an important genital pathogen. (Am J Obstet Gynecol 1985;151:338-9.)

Key words: *Haemophilus influenzae,* salpingitis

Haemophilus influenzae has rarely been isolated directly from the fallopian tubes.[1] We report two cases of women with pelvic inflammatory disease who had serologically nontypable *H. influenzae* but no other microorganisms isolated from the fallopian tubes at laparoscopy.

Case reports

Case 1. A 22-year-old, gravida 0, para 0 woman was admitted to the hospital because of pelvic pain of 7 days' duration. Her last sexual exposure was 21 days earlier, and she practiced oral sex. On examination, mucopurulent endocervical discharge and moderate uterine and left adnexal tenderness were noted. The erythrocyte sedimentation rate was 53, and white blood cell count was 13.4×10^9/L. A Papanicolaou cervicovaginal smear showed coccal flora and a large number of endocervical leukocytes (3+) but no cellular atypia. Since acute pelvic inflammatory disease was suspected, laparoscopy was performed the next day, after removal of the intrauterine contraceptive device. Laparoscopy showed severe salpingitis with a yellow discharge coming out from the fimbrial end of the right fallopian tube. A left pyosalpinx was punctured under laparoscopic control and thick yellow pus was obtained. *H. influenzae* (3+ growth) but no other microorganism was isolated from both fallopian tubes. The isolated strain was serologically nontypable *H. influenzae,* Kilian's biotype II (Table I). *H. influenzae* (same strain, 3+ growth) together with *Bacteroides bivius,* α-hemolytic streptococci, and *Chlamydia trachomatis* was isolated from the endometrial aspirate obtained transcervically by means of a double-lumen catheter. Except for the isolation of *Trichomonas vaginalis* from the vagina and *C. trachomatis* from the endometrium, cultures for *C. trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, T. vaginalis, Ureaplasma urealyticum,* herpes simplex virus, and cytomegalovirus were negative from all sites (cervix, endometrium, fallopian tubes, and cul-de-sac). Cytologic examination of peritoneal fluid revealed moderate nonspecific inflammation with a large number (3+) of polymorphonuclear leukocytes. Endometrial biopsy showed severe plasma cell endometritis with lymphoid follicles. Following laparoscopy, intravenous treatment with doxycycline, 100 mg twice daily, and metronidazole, 500 mg three times daily, for 2 days was started. Treatment continued orally for 2 weeks and resulted in uneventful recovery.

Case 2. A 29-year-old, gravida 1, para 1 woman was admitted to the hospital because of fever, low abdominal pain, and intermenstrual vaginal bleeding of 7 to 10 days' duration. Her last sexual exposure was 14 days earlier, and she denied oral sex. On examination, purulent cervicovaginal discharge, moderate cervical motion tenderness, and severe adnexal tenderness were noted. The erythrocyte sedimentation rate was 64, and white blood cell count was 12.8×10^9/L. At laparoscopy severe salpingitis with left tuboovarian abscess was seen, and 40 ml of yellow pus was aspirated from the abscess. Severe erythema of the uterus and filmy avascular adhesions involving the cul-de-sac were seen. *H. influenzae* (3+ growth) was isolated from the tubal aspirate but not from the endometrium or cul-de-sac. The strain was serologically nontypable *H. influenzae,* biotype III (Table I); cervical, endometrial, fallopian tube, and cul-de-sac cultures for *N. gonorrhoeae, C. trachomatis,* cytomegalovirus, herpes simplex virus, *T. vaginalis, M. hominis,* and *U. urealyticum* were all negative. Papanicolaou smear showed benign inflammatory atypia of the epithelial cells and was positive for clue cells and actinomyces-like organisms. Cytologic examination of the peritoneal fluid revealed a large number of polymorphonuclear leukocytes. Endometrial biopsy showed moderate plasma cell endometritis. Intravenous treatment with aqueous penicillin G procaine, 3 MU four times daily, and metronidazole, 500 mg three

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Table I. Characteristics of the two *H. influenzae* strains isolated from patients with acute salpingitis and two reference strains

Strain	*Serotype**	*V-factor requirement*	*Utilization of δ-alanine*	*Formation of indole*	*Urease activity*	*Ornithine-decarboxylase activity*	*Biotype of isolated strain*
H. influenzae (ATCC 35056)	NT	+	–	+	+	+	I
H. influenzae (NCTC 8468)	NT	+	–	–	+	+	IV
H. influenzae (Case 1)	NT	+	–	+	+	–	II
H. influenzae (Case 2)	NT	+	–	–	+	–	III

*Serotyping was done by use of a commercial latex-agglutination lot (Difco). NT, Nontypable.

times daily, was started followed by oral treatment for 2 weeks. On examination 2 weeks later, ultrasonography revealed an echo-dense mass on both sides of the uterus. Two weeks later the examination was normal.

Comment

Both patients had mucopurulent cervicitis, endometritis (determined by endometrial biopsy), and salpingitis (seen at laparoscopy), indicating intraluminal infection. We have so far examined 36 patients with suspected pelvic inflammatory disease, of whom 29 had salpingitis at laparoscopy. Thus the isolation rate of *H. influenzae* from the fallopian tubes of women with salpingitis was 7% (two of 29). None of the case subjects or control subjects who did not have salpingitis had *H. influenzae* isolated from the tubes (or from the endometrium). The negative culture results for other organisms from the fallopian tubes in these two cases can hardly be explained by insensitivity or failure of the culture techniques since many other organisms have been isolated from the tubes in other cases of pelvic inflammatory disease in this ongoing study. Although *C. trachomatis* and α-hemolytic streptococci were simultaneously isolated from the endometrium of the first patient (Case 1), only *H. influenzae* was isolated from the tubes.

It was of particular interest that both of the isolated strains belonged to the serologically nontypable group of *H. influenzae* which has been suggested to play a role in the infections affecting this area.[2] The source of the isolated *H. influenzae* strains could not be determined as urethral and pharyngeal cultures from the sex partners of the two patients were not obtained.

Our study indicates that the serologically nontypable strain of *H. influenzae* should be included among the microorganisms which can cause purulent infection of the fallopian tubes.

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Malformations and chromosome anomalies in spontaneously aborted fetuses with single umbilical artery

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Presence of a single umbilical artery was noted 13 times among a series of 879 consecutively collected, spontaneously aborted embryos and fetuses. This rate of 1.5% is approximately twice that reported from the largest series of term births. Eight of the 13 fetuses had additional serious malformations. Of nine successfully karyotyped fetuses, six had chromosome anomalies. All six occurred in fetuses with severe malformations in addition to the single umbilical artery. The presence of single umbilical artery with other major anatomic defects may be a significant predictor of the presence of chromosome anomalies in live births. Early recognition of offspring with chromosome anomalies will be useful in counseling the family and in managing the care of the infant. (Am J Obstet Gynecol 1985;151:340-2.)

Key words: Single umbilical artery, fetus, karyotype, spontaneous abortion

The association between the absence of one of the two umbilical arteries and the presence of other malformations has been known since the time of the Renaissance, and has been the subject of numerous studies in the past 30 years. Most recent reports have been concerned with infants and stillbirths; few authors have studied this phenomenon in fetal deaths prior to 28 weeks. Furthermore, chromosome studies have been carried out only sporadically within the existing studies. This paper describes the association between the presence of a single umbilical artery, other malformations, and chromosome anomalies in a consecutively collected series of spontaneous abortions.

Material and methods

The data are derived from an epidemiologic, cytogenetic, and pathologic study of spontaneous abortions conducted between January, 1977, and August, 1981, in New York City[1] (also unpublished observations). The identification of single umbilical artery was made by inspection of the cord, by inspection of the pelvic aortic branches, by histologic examination of the cord, or by a combination of all three methods.

Although nearly 3500 specimens from spontaneous abortions were examined over the study period, the frequency of single umbilical artery is based only on those cases whose development had proceeded at least to the point where the number of umbilical vessels and the presence of malformations could be detected. About three quarters of the total number of specimens were excluded by this criterion, which left 879 cases of spontaneous abortions consisting of intact embryos >10 mm crown-rump length and all fetuses, by definition >30 mm crown-rump length.

Results

Presence of a single umbilical artery was identified in 13 individuals among 879 singleton embryos and fetuses. The rate of single umbilical artery in this series of spontaneous abortions is therefore 14.8 per 1000 embryos and fetuses (1.5%). Eight of the 13, or 62%, had additional malformations. Table I lists the 13 individual cases with their other associated malformations and karyotypes.

In four cases, single umbilical artery occurred as an isolated malformation; one additional specimen also had a malrotation of the gut. The remainder had a variety of malformations, all of them severe. Nine fetuses were successfully karyotyped; three had a normal karyotype and six had chromosome anomalies—three cases of trisomy 18 and one each of trisomy 7, triploidy, and monosomy X. The three normal karyotypes occurred in fetuses without other severe malformations. No fetus with single umbilical artery and severe malformations had a normal karyotype. The rate of chromosome anomalies in fetuses with single umbilical artery, 67%, is significantly higher ($X_1^2 = 4.07$; $p < 0.05$) than the rate of chromosome anomalies in fetuses with other malformations, which was 31% (24/74) (unpublished observations).

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Table I. Spontaneously aborted embryos and fetuses with single umbilical artery

No.	*Karyotype*	*Week of gestation*	*Crown-rump length (mm)*	*Sex*	*Description*
1	46,XX	16	94	F	Single umbilical artery
2	—	27	200	F	Single umbilical artery
3	46,XX	23	120	F	Single umbilical artery, malrotation of the gut
4	—	17	—	—	Single umbilical artery
5	46,XX	21	163	F	Single umbilical artery
6	—	—	75	M	Single umbilical artery, short arms and legs
7	—	28	155	M	Single umbilical artery, arthrogryposis, malrotation of the gut, ectopic gonad
8	69,XXX	23	135	F	Single umbilical artery, hydrocephalus, ventricular septal defect, adrenal and pulmonary hypoplasia, ambiguous genitalia, small kidney, syndactyly of hands (R, 3 + 4; L, 2 + 3 + 4) and feet (R + L, 2 + 3 + 4 + 5)
9	47,XY, − 18	27	210	M	Single umbilical artery, talipes, microcardia, hypoplastic lungs, malrotation of the gut, enlarged urinary bladder, micropenis, prune belly, syndactyly of left foot (3 + 4)
10	47,XY, + 18	28	192	M	Single umbilical artery, cleft lip and palate, ventricular septal defect, hydrocephalus, small chin and eyes, malformed ears, hypoplastic lungs, malrotation, left renal agenesis, omphalocele, polydactyly, syndactyly
11	47,XX, + 18	—	55	F	Single umbilical artery, short arms and legs, cleft lip and palate
12	47,XY, + 7	20	100	M	Single umbilical artery, cleft palate, hypoplastic mandible, right descending aorta, malrotation, small kidney and adrenal, micropenis
13	45,X	25	195	F	Single umbilical artery, short arms and legs, ventricular septal defect, bicuspid aortic valve, persistent left superior vena cava, preductal coarctation, hypoplastic lungs, horseshoe kidney, asymmetric gonads, cystic hygroma

Comment

The rate of occurrence of single umbilical artery in our study, 1.5%, is twice as high as rates reported from the largest unselected series of term births. Bryan and Kohler[2] reported a rate of 0.72% from nearly 20,000 placentas, and Heinonen et al.[3] found single umbilical artery in 0.75% of 45,192 children in the Collaborative Perinatal Project. Other studies have found rates closer to 1%.[4-6] The only other study to have reported on the rate of single umbilical artery in spontaneous abortions[7] found this condition in four of 148 fetuses, for a rate of 2.7%. The differences between this rate and that found in our study may reflect real spatial and/or temporal differences, may represent random variation, or may be due to a sampling technique that overemphasized malformed fetuses.

The rate of other malformations in infants with single umbilical artery is about 18% in the three largest series.[2,3,8] In our series 62% of cases of single umbilical artery had other serious malformations, an increase of about 3.5 times over the rate at term. In our study, we found no preponderance of anomalies of any one organ system. This was true of the Collaborative Perinatal Project also, where the risks for having another malformation if the infant had single umbilical artery ranged from 5.1 for defects of the cardiovascular system to 10.8 for malformations of the genitourinary system, excluding hypospadias.[3]

It is noteworthy that in our series all fetuses which had both single umbilical artery and major malformations also had chromosome anomalies. The absence of one umbilical artery in infants with chromosomal syndromes has been described by a number of authors (reviewed in Reference 2). Trisomy 18 has been the subject of the most numerous reports, but trisomy 13, trisomy 21, and Turner's syndrome, or monosomy X, have also been reported with single umbilical artery.[2] Single umbilical artery was identified in one of three spontaneously aborted fetuses with monosomy X by Singh and Carr.[9] It is not known how many term infants with single umbilical artery with or without other malformations would have chromosome anomalies if all such infants were karyotyped. Vlietinck et al.[6] successfully karyotyped 19 of 29 consecutively identified infants with single umbilical artery and found no chromosome anomalies among them. In another study Lenoski and Medovy[10] found normal chromosomes in three otherwise anatomically normal infants with single umbilical artery.

In our series of 879 embryos and fetuses 10.3% of malformed fetuses and 17.1% of chromosomally abnormal fetuses had single umbilical artery. Three of six cases of trisomy 18 had single umbilical artery, as did the single trisomy 7 case. Single umbilical artery also occurred in one of three triploid fetuses and in one of four fetuses with monosomy X. None of eight fetuses with trisomy 21 had single umbilical artery. It is possible that the wide range of malformations associated with single umbilical artery is explained by its association with chromosome anomalies, many of which have multiple anatomic defects.

This small series has shown that the rate of single umbilical artery among early fetal deaths exceeds the rate at term by about twofold, and the rate of other malformations among affected fetuses is about three times higher than the comparable rate in term births. In our study all fetuses with single umbilical artery and other major malformations had chromosome anomalies—trisomies, triploidy, and monosomy X.

If results from studies of fetuses can be extrapolated to term births, then our study suggests that the association of single umbilical artery with chromosome anomalies among malformed term births may be stronger than has previously been supposed. If further studies bear out this hypothesis, then karyotyping of newborns where single umbilical artery occurs in combination with other malformations should become part of standard obstetric practice. Early recognition of syndromes involving chromosome anomalies will be useful for counseling parents and for management decisions regarding the infant.

Prenatal identification by means of ultrasonography of fetuses affected with single umbilical artery[11] should alert clinicians to the need for a more extensive search for other malformations and subsequent cytogenetic studies should they be found.

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Fetal assessment based on fetal biophysical profile scoring: Experience in 12,620 referred high-risk pregnancies

I. Perinatal mortality by frequency and etiology

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Fetal biophysical profile scoring was used as a method for antepartum fetal risk assessment in 12,620 high-risk patients referred in a 55-month interval. A total of 26,257 tests were performed on these patients (range, one to 18 tests per patient). Ninety-three perinatal deaths occurred (gross perinatal mortality rate, 7.37 per 1000) of which 62 (66.6%) were due to a major anomaly, seven were due to Rh disease (7.5%), and the remaining 24 deaths (25.8%) occurred in structurally normal fetuses. The corrected perinatal mortality rate was 1.90 per 1000. Eight structurally normal fetuses died within 1 week of a normal test result (corrected false negative rate, 0.634 per 1000). These data suggest fetal biophysical profile scoring is an accurate method for identification of the fetus at risk for perinatal death. (AM J OBSTET GYNECOL 1985;151:343-50.)

Key words: Fetal biophysical profile, high-risk pregnancies, perinatal mortality

Accurate prenatal recognition of acquired and congenital disease states that affect fetal function and structure must be a first critical step in reducing or preventing perinatal morbidity and mortality. The advent of real-time ultrasound imaging now permits simultaneous assessment of a wide range of fetal structural and functional characteristics. In previous reports we and others have noted an improvement in perinatal mortality when five discrete fetal biophysical indices were observed and combined (fetal biophysical profile score) for use in perinatal management decisions.[1-4] In this prospective clinical study of 12,620 referred high-risk pregnancies further experience with this method and the resultant perinatal mortality are described. Perinatal mortality, considered in whole and by component parts, is selected as the end point to judge efficacy of the method since it is not subject to observer bias and may therefore permit objective comparison between this and other methods and between this and other centers. Perinatal morbidity as observed in this study will be detailed in a subsequent report.

Methods

The study period was for 55 consecutive months ending January, 1984. During this period consecutive referred patients with recognized high-risk factors (Table I) and at a gestational age of at least 26 weeks were entered into the study. Patients with a fetal death recognized at initial referral (71 patients) were excluded from the study population. A fetal biophysical profile score was recorded in all patients at all visits. In all patients, at the initial visit and most subsequent visits, the ultrasound examination included a detailed anatomic screen for anomalies and recording of fetal morphometric data.

The method used for fetal biophysical profile scoring has been reported previously (Table II).[5] Four variables (fetal movement, fetal breathing, fetal tone, and qualitative amniotic fluid volume) were observed simultaneously during ultrasound examination; the duration of observation was extended until each ultrasound-monitored variable met normal criteria or 30 minutes elapsed. In most instances (97%) when variables were normal the duration of ultrasound observation was <10 minutes. Variables were assigned an arbitrary score of 2 when normal and 0 when abnormal. In the first 22 months of the study a nonstress test was done in all patients regardless of the results of the initial ultrasound observation. Interval analysis of the data indicated that the addition of the nonstress test did not improve discriminative accuracy when all ultrasound-monitored variables were recorded as normal.[6] Based

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Table I. Distribution of study population by primary indication* for referral

Indication	*No. of patients*	*% of total*
Suspected intrauterine growth retardation	2625	20.8
Hypertension	2209	17.5
Postdates (42 weeks)	1464	11.6
Diabetes (total)	1161	9.2
Class A	909	7.1
Insulin-dependent	252	2.1
Previous low-segment cesarean section	618	4.9
Antepartum hemorrhage	568	4.5
Decreased fetal movement	543	4.3
Previous stillbirth	542	4.3
Maternal disease	441	3.5
Twins	416	3.3
Suspected anomaly	341	2.7
Premature labor	303	2.4
Premature rupture of membranes	278	2.2
Large for dates	177	1.4
Fetal heart rate anomaly	175	1.4
Rh disease	139	1.1
Other	620	4.9
Total	12,620	100

*Many patients had multiple risk factors. Classification is by the factors promoting initial referral.

on this analysis, routine nonstress testing was discontinued when the ultrasound variables were normal and used only when one or more of the ultrasound-monitored variables was abnormal. Additional tests of fetal well-being such as maternal serum estriol levels were not used at all and contraction stress testing was used rarely.

Perinatal management decisions were based on the results of the fetal biophysical profile score always as considered within the overall clinical context of each case (Table III). A normal score (biophysical profile score ≥ 8) was considered as indicative of a nonasphyxiated fetus and in such cases the indication for delivery, if any, was based on other fetal, maternal, or obstetric factors. Thus, for example, delivery was recommended in the presence of a normal score if the fetus was recognized as being growth retarded and pulmonary maturity was confirmed, if the patient was beyond 42 completed weeks of gestation and the cervix was favorable for induction of labor, or if the maternal medical condition (for instance, preeclampsia) was deteriorating. Further, when oligohydramnios was observed in the postdate patient delivery was recommended even if the other components of the biophysical profile score were normal. An equivocal test result (biophysical profile score = 6) was considered as an indication for repeat testing within 24 hours and if results were equivocal or abnormal at repeat testing then delivery was recommended. An abnormal test result (biophysical profile score ≤ 4) was always considered an indication for delivery, and management was delayed only in the presence of extreme fetal immaturity (<28 completed weeks of gestation). In all instances in which delivery was recommended, the route of delivery was based on a consideration of fetal, obstetric, and maternal factors; a trial of vaginal delivery was recommended unless otherwise contraindicated.

Perinatal mortality was defined as death of a perinate of a birth weight of at least 500 gm and included deaths occurring within 28 completed days after birth. All perinatal deaths were assigned to one of three categories, based upon probable cause of death. The groups were: (1) death due to asphyxia or unknown cause in an otherwise structurally normal perinate, (2) death as a direct result of major anomaly(s), and (3) death as a consequence of Rh isoimmunization including traumatic death at the time of intrauterine transfusion. The latter group is defined separately since this center is a major referral center for treatment of isoimmunized fetuses and as a result such cases contribute disproportionately to the overall mortality.[7] The false negative rate in this study is defined as stillbirth of a structurally normal fetus within 1 week of a last normal fetal biophysical score (≥ 8).

Results

In total, 12,620 consecutive high-risk patients with a live fetus at the time of initial referral were studied. An additional 71 patients were noted to have a fetal death at the time of initial assessment and were not considered as part of the study group. In the study group the mean gestational age at the time of initial assessment was 34.3 ± 1.8 weeks (range, 26 to 44 weeks). The distribution of these study patients in accordance with the primary indication for referral is listed in Table I; most patients had more than one risk factor at initial referral. It is noted that four major risk factors accounted for more than half the study population (59.1%), being suspected intrauterine growth retardation (20.8%), hypertensive disorders of pregnancy (17.5%), postdate pregnancy (11.6%), and diabetes (9.2%).

In total, there were 26,257 fetal biophysical profile tests performed in the 12,620 patients (mean, 2.1 per patient; range, one to 18 tests). Distribution of test by score is listed in Table IV. Most tests (25,606 tests, 97.52%) were scored as normal (biophysical profile score ≥ 8). A total of 452 tests (1.72%) demonstrated the absence of two variables (equivocal, biophysical profile score = 6), and the remaining 199 tests (0.76%) had three or more variables absent (biophysical profile score ≤ 4). Most equivocal tests (82%) were normal on repeat testing within 24 hours. Conversion of an abnormal

Table II. Biophysical profile scoring: Technique and interpretation*

Biophysical variable	*Normal (score = 2)*	*Abnormal (score = 0)*
1. Fetal breathing movements	$\geq$1 episode of $\geq$30 sec in 30 min	Absent or no episode of $\geq$30 sec in 30 min
2. Gross body movements	$\geq$3 discrete body/limb movements in 30 min (episodes of active continuous movement considered as single movement)	$\leq$2 episodes of body/limb movements in 30 min
3. Fetal tone	$\geq$1 episode of active extension with return to flexion of fetal limb(s) or trunk. Opening and closing of hand considered normal tone	Either slow extension with return to partial flexion or movement of limb in full extension or absent fetal movement
4. Reactive fetal heart rate	$\geq$2 episodes of acceleration of $\geq$15 bpm and of $\geq$15 sec associated with fetal movement in 20 min	<2 episodes of acceleration of fetal heart rate or acceleration of <15 bpm in 20 min
5. Qualitative amniotic fluid volume	$\geq$1 pocket of fluid measuring $\geq$1 cm in two perpendicular planes	Either no pockets or a pocket <1 cm in two perpendicular planes

*See reference 5.

Table III. Biophysical profile scoring: Management protocol

Score	*Interpretation*	*Recommended management*
10	Normal infant, low risk for chronic asphyxia	Repeat testing at weekly intervals. Repeat twice weekly in diabetic patients and patients $\geq$42 wk
8	Normal infant, low risk for chronic asphyxia	Repeat testing at weekly intervals. Repeat twice weekly in diabetic patients and patients $\geq$42 wk. Indication for delivery = oligohydramnios
6	Suspected chronic asphyxia	Repeat testing within 24 hr. Indication for delivery = oligohydramnios or remaining $\leq$6
4	Suspected chronic asphyxia	$\geq$36 score and favorable cervix. If <36 wk and lecithin/sphingomyelin ratio <2.0, repeat test in 24 hr. Indication for delivery = repeat score $\leq$6 or oligohydramnios
0-2	Strong suspicion of chronic asphyxia	Extend testing time to 120 min. Indication for delivery = persistent score $\leq$4, regardless of gestational age

score (biophysical profile score $\geq$4) to normal (biophysical profile score $\geq$8) occurred in only one instance (0.5%).

Ninety-three perinatal deaths occurred among the study population for a gross (uncorrected) perinatal death rate of 7.37 per 1000 live births. Forty-six of these deaths were stillbirths (49.5%) and 47 were neonatal deaths (50.5%). The gross stillbirth rate was 3.64 per 1000 and the gross neonatal death rate was 3.72 per 1000. Perinatal mortality as related to test score result is listed in Table IV; mortality ranged from as low as 0.652 per 1000 tests with a normal score (biophysical profile score = 8) to as high as 187 per 1000 tests with a completely abnormal score (biophysical profile score = 0). Total perinatal deaths classified by cause are listed in Table V. In total, 69 of 93 (74.2%) perinatal deaths were a direct result of either major lethal anomalies (66.7%) or severe Rh disease (7.5%). Perinatal morbidity is considered in a separate report to follow.

Perinatal deaths directly attributable to a major anomaly were classified according to the major systems affected (Table VI); anomalies affecting at least two major systems were classified as multiple anomalies. In total, 62 of 93 perinatal deaths (66.7%) were due to a major anomaly, of which 19 were major central nervous system anomalies (30.6%), 11 were major renal anomalies (17.6%), nine were major gastrointestinal anomalies (13.2%), eight were major cardiac anomalies (13%), seven were major skeletal anomalies (11.2%), seven were multiple anomalies (11.2%), and one anomaly was due to a major cytogenetic disorder (1.6%). Seven deaths were a direct result of Rh isoimmunization, four of which were due to trauma at the time of intrauterine transfusion and three occurred within 48 hours of intrauterine transfusion and were considered the result of advanced fetal disease (hydrops fetalis).

The remaining 24 deaths, representing 25.8% of the total deaths, occurred among structurally normal fetuses and therefore all may be considered as potentially preventable. Thus, the corrected perinatal mortality

Table IV. Test score distribution and associated corrected perinatal mortality

Test score result	*No. of tests*	*% of total tests*	*Corrected perinatal mortality** *No. of deaths*	 *Rate per 1000 tests*
8-10 (normal)	25,606	97.52	16	0.652
6 (equivocal)	452	1.72	0	0
4 (abnormal)	136	0.52	3	22.0
2 (abnormal)	47	0.18	2	42.6
0 (abnormal)	16	0.06	3	187
Total	26,257	100	24	—

*Excludes deaths due to either major congenital anomaly or severe Rh isoimmunization.

Table V. Perinatal deaths by cause and time of death (n = 93)

Category	*Stillbirths* *No. of deaths*	 *% of total*	*Neonatal deaths* *No. of deaths*	 *% of total*	*Total all deaths* *No. of deaths*	 *% of total*
Major (lethal) anomaly	26	56.6	36	76.6	62	66.7
Rh disease/Rh trauma	5	10.8	2	4.3	7	7.5
Death in normal fetus	15	32.6	9	19.1	24	25.8
Total	46	100	47	100	93	100

Table VI. Perinatal death due to major anomaly: Classification by anomaly type (n = 62)

Anomaly type	*Stillbirths*	*Neonatal deaths*	*Total*
Major central nervous system (total)	9	10	19
Anencephaly/neural tube	7	8	
Holoprosencephaly	1	2	
Hydrocephalus	1		
Major renal (total)	6	5	11
Renal agenesis	5	1	
Obstructive uropathy	1	4	
Major gastrointestinal (total)	1	8	9
Diaphragmatic hernia	0	4	
Omphalocele/cardiac	1	1	
Other	0	3	
Major cardiac (total)	0	8	8
Ebstein's anomaly	0	1	
Ventricular septal defect	0	3	
Hypoplastic ventricles	0	2	
Other	0	2	
Major skeletal dystrophy	7	0	7
Thanatophoric dwarfism	2	0	
Achondroplastic	2	0	
Unclassified	3	0	
Major multiple anomalies (total)	3	4	7
Major chromosomal anomalies (total)	1	0	1
Total	27	35	62

rate in this study population was 1.90 per 1000. Fifteen of these 24 deaths (62.5%) occurred as stillbirths, for a corrected stillbirth rate of 1.18 per 1000. The relationship of interval between testing and stillbirth and the last test result before stillbirth is listed in Table VII. Ten stillbirths occurred within 1 week of the last test (range, 2 to 7 days); of these, eight occurred following a *normal* last test result. Therefore, the false negative rate was 0.634 per 1000. In three of these false negative cases the deaths occurred in fetuses of diabetic patients; in two the cause of death was unexplained and one death was the result of abruptio placentae. In three false negative cases death occurred among fetuses of patients being evaluated for intrauterine growth retardation; the diagnosis was confirmed in two of these infants at delivery, but in the remaining case no evi-

Table VII. Stillbirths in structurally normal fetuses without Rh isoimmunization as classified by test-to-death interval and last test result (n = 15)

Patient	*Interval (days)*	*Last test result*	*Clinical diagnosis*
Interval of ≤7 days, last test normal (≥8) (n = 8)			
V. H.	3	8 (NST not done)	Suspected IUGR, 34 wk
V. L.	5	10 (NST reactive)	Suspected IUGR, 32 wk
J. R.	5	8 (NST not done)	Postdate, 42 wk
A. T.	5	8 (NST not done)	Class B diabetes/massive abruptio placentae, 38 wk
V. D.	6	10 (NST reactive)	Hypertension, 39 wk
J. M.	7	8 (NST reactive)	Suspected IUGR, 36 wk
C. S.	7	8 (NST not done)	Class B diabetes, 35 wk
M. R.	7	8 (NST not done)	Class A diabetes, 37 wk
Interval of >7 days, last test normal (≥8) (n = 3)			
E. C.	15	8 (NST not done)	Suspected IUGR, 34 wk
M. M.	21	8 (NST not done)	Suspected IUGR, 29 wk, abruptio placentae
S. C.	28	8 (NST not done)	Multiple sclerosis, 29 wk
Interval of ≤7 days, last test abnormal (≤4) (n = 2)			
L. C.	2	4 (absent FBM and FM, (NST nonreactive)	Hypertensive at term, intrapartum death—not monitored in labor
D. S.	5	4 (absent FBM and FT, oligohydramnios)	Delivery delayed, <27 wk
Interval of >7 days, last test abnormal (≤4) (n = 2)			
C. S.	11	0	IUGR, delivery delayed because of immaturity (26 wk)
C. M.	11	2 (fetal movement only)	IUGR, delivery delayed because of immaturity (<29 wk)

NST: Nonstress test; IUGR: intrauterine growth retardation; FBM: fetal breathing movements; FM: fetal movements; FT: fetal tone.

dence of intrauterine growth retardation was determined at delivery. The remaining two deaths were associated with maternal hypertension and postmaturity, respectively. Two stillbirths occurred within 1 week of an *abnormal* last test result. In one case a score of 4 was recorded in a hypertensive patient at 38 weeks and medical induction of labor was suggested but was delayed for 48 hours. The patient went into spontaneous labor and intrapartum fetal heart rate monitoring was not used; the fetus died during the intrapartum period. In the other case a biophysical profile score of 4 was recorded in a growth-retarded fetus at 27 weeks' gestation and delivery delayed because of fetal immaturity; fetal death occurred 5 days later. Five stillbirths occurred at an interval >7 days after the last normal fetal biophysical profile score (range, 11 to 28 days).

Two stillbirths occurred >7 days after a last abnormal test result. In both, severe intrauterine growth retardation was recognized, but delivery was delayed because of the extreme fetal immaturity (28 and 26 weeks, respectively). It is of interest to note that in both of these cases fetal death did not occur until 11 days after the last abnormal score.

Nine neonatal deaths occurred in structurally normal neonates (corrected neonatal death rate, 0.72 per 1000, Table VIII). In five of these deaths the last fetal biophysical profile score before delivery was normal; four of these deaths were due to respiratory distress syndrome in fetuses whose birth weight was <1500 gm (range, 830 to 1460 gm) and one death was due to intrapartum chorioamnionitis and neonatal sepsis (birth weight, 4520 gm). The remaining four neonatal deaths occurred after a last fetal biophysical profile score that was abnormal. In two of these deaths the last score before delivery was 0. One was recognized as having severe intrauterine growth retardation at the first visit at 37 weeks; despite immediate delivery by emergency low-segment cesarean section, the fetus was grossly asphyxiated at birth and died. The other death occurred in a 27-week infant whose mother was severely hypertensive. One death occurred as a result of meconium aspiration in a term infant after a last test score of 4, and one death occurred in an infant with a last test score of 2 and was due to pulmonary immaturity (respiratory distress syndrome).

Comment

The natural relationship between a fetal biophysical profile score as used in this study and perinatal mortality has been reported previously; in the absence of intervention, perinatal mortality rose progressively as the fetal biophysical profile score fell.[5] In this current series of 12,620 referred high-risk pregnancies in which antepartum fetal assessment and obstetric management decisions were based on fetal biophysical profile scoring, we observed 93 perinatal deaths, for a mor-

Table VIII. Neonatal deaths among structurally normal infants (n = 9)

Case No.	*Patient*	*Last test score*	*Interval from test to delivery*	*Birth weight (gm)*	*Cause of death*
Last test score normal					
1	I. M.	10 (reactive NST)	1 day	3860	Choriamnionitis, sepsis
2	M. B.	8 (NST not done)	7 days	1100	Respiratory distress syndrome, hyaline membrane disease
3	S. J.	10 (reactive NST)	7 days	1460	Respiratory distress syndrome, hyaline membrane disease
4	S. Y.	8 (NST not done)	10 days	1100	Respiratory distress syndrome, hyaline membrane disease
5	M. J.	8 (NST not done)	10 days	860	Respiratory distress syndrome, hyaline membrane disease
Last test score abnormal					
6	C. S.	0	<1 hr	1860	Severe asphyxia, intrauterine growth retardation
7	E. K.	0	2 days	770	Asphyxia, respiratory distress syndrome, hyaline membrane disease
8	C. L.	4 (absent FM, FBM, nonreactive NST)	3 days	4520	Meconium aspiration
9	M. L.	2 (absent FM, tone, nonreactive NST, absent FBM)	6 days	760	Respiratory distress syndrome, hyaline membrane disease

tality rate of 7.37 per 1000. This death rate is similar to that reported with the method in smaller study populations from this and other centers.[1-4] As the causes and mechanisms of perinatal death are of diverse origin, it is likely that multiple factors may be operant in explaining the remarkably low mortality rate observed. Since matched nontested control subjects were not available, the extent of change in observed from expected mortality may be estimated by indirect methods only. During the study interval within the catchment area population there were 65,979 deliveries, the majority of which (80%) were classified as low risk by maternal risk screening methods.[8,9] Within this entire population there were 943 perinatal deaths, for a perinatal mortality rate of 14.3 per 1000.[7] The perinatal mortality rate in the study population (7.37 per 1000), all of whom had high-risk pregnancies (Table I), was reduced to nearly one half (48.5%) that rate observed in this general population. The gestational age at which antepartum testing is begun may influence mortality statistics since the death rate due to immaturity will decline as the gestational age at first test advances. In this study population the mean gestational age at which testing was begun, 34.3 weeks, almost certainly is a major factor in the low neonatal death rate observed in the absence of major anomaly (0.72 per 1000). Analysis of the frequency and cause of stillbirth may be a better method of judging the efficacy of the fetal biophysical profile scoring since maturity factors are less significant. In the study population 46 stillbirths were reported (stillbirth rate, 3.64 per 1000), of which 15 stillbirths (32.6%) occurred in structurally normal, nonisoimmunized fetuses (corrected stillbirth rate, 1.18 per 1000). In contrast within the entire population during the study interval there were 477 reported stillbirths (stillbirth rate, 7.22 per 1000), of which 419 (88%) occurred in structurally normal fetuses (corrected stillbirth rate, 6.35 per 1000).[8] These comparisons, although of a general nature, suggest that within the study population a true decrease of undetermined magnitude has occurred in the perinatal death rate, the stillbirth rate, and the corrected stillbirth rate. Further, such comparisons suggest that in the study population the decreased stillbirth frequency is associated with a change in etiology such that the relative number of deaths due to anomaly may be increased substantially, that is, to 56.6% in the study population as compared to 12% in the population at large. These data suggest that the low stillbirth rate observed in the study population is largely due to a decrease in deaths among structurally normal fetuses.

Antepartum recognition of fetal asphyxia by the use of fetal biophysical profile scoring contains inherent inaccuracies. In this study we recorded eight intrauterine deaths in structurally normal fetuses within 1 week of a last normal test result (corrected false negative rate, 0.634 per 1000). This observed false negative rate with fetal biophysical profile scoring is similar to that reported for contraction stress testing (0.4 per 1000) and lower than that reported with the nonstress test (3.2 per 1000).[10] Neither of these latter methods of fetal assessment based on antepartum fetal heart rate monitoring reliably detect major anomalies whereas anomaly detection is an inherent part of the ultrasound-based fetal biophysical profile scoring method.[1, 11]

The positive predictive accuracy of an abnormal test (biophysical profile score ≤4) in predicting perinatal mortality and the natural interval between abnormal score and death cannot be determined in this study since by protocol an abnormal score (biophysical profile score ≤4) in a structurally normal fetus was an indication for delivery. However, in four cases of stillbirth, delay occurred between the observation of an abnormal score and intervention (Table VIII). In one case (L. C.) the referring physician elected to delay delivery; the patient returned in spontaneous labor 2 days after a score of 4 and a nonmonitored intrapartum death ensued. In the other three cases delivery after an abnormal score was delayed because of prematurity (<29 weeks). Fetal death occurred as early as 5 days and as late as 11 days after the abnormal score. We were surprised to note an interval of 11 days between a score of 0 and fetal death in one patient with extreme prematurity (C. S., Table VIII). In contrast, in one neonatal death (C. S., Table VIII), recognition of a score of 0 prompted delivery within the hour, but despite aggressive resuscitation, the newborn infant remained severely asphyxiated and died within 24 hours of birth.

The distribution of test score results in this study indicated that the vast majority of tests were normal (97.5%) and that equivocal or abnormal test results were unusual (1.72% and 0.76%, respectively, Table IV). This distribution differs sharply from test result distributions reported for contraction stress testing (84.6% normal)[12, 13] and nonstress testing (89% normal).[14] This altered test result distribution with fetal biophysical profile scoring may indicate an improved ability to differentiate the normal fetus from the compromised one. Since the false negative rate with fetal biophysical profile scoring is as low as that reported for the contraction stress test and lower than that reported for the nonstress test, these distribution data suggest that the method may be a better alternative to either antepartum fetal heart rate monitoring method.

The concept of fetal biophysical profile scoring involves evaluation of immediate biophysical activities (fetal movement, tone, breathing movements, and heart rate activity) together with semiquantitative determination of amniotic fluid. The first four of these variables reflect acute central nervous system function and, when they are present, it is reasonable to deduce that central nervous system depression (for instance, asphyxia) is not present.[15-17] In our expanded experience we note that the same accuracy of prediction of normal is maintained when only three of these acute variables (fetal movement, tone, and breathing) are considered.[6, 18] The absence of a given variable (for instance, fetal breathing movements) is more difficult to interpret since central nervous system–regulated periodicity for these variables is recognized.[19] Extension of the observation to at least 30 minutes is required before an acute variable may be considered absent on a pathologic basis. The absence of three or more short-term variables in a 30-minute period strongly suggests fetal compromise. In this study of 26,257 tests, we observed only one instance of an abnormal score (biophysical profile score ≤4) reverting to normal (biophysical profile score ≥8). In this case the fetus sustained major head trauma associated with a maternal pelvic fracture and had an initial score of 2 (normal amniotic fluid). Over the course of 3 weeks the score became normal; the child was not asphyxiated at delivery and survived. In contrast to the other profile variables, semiquantitative amniotic fluid volume may be considered as a marker of chronic fetal compromise. In the presence of intact membranes we have shown that oligohydramnios develops as a gradual process over a period of days, and when oligohydramnios is present the risk of perinatal asphyxia and mortality rises sharply.[20] The mechanism by which fetal asphyxia results in oligohydramnios is not fully elucidated but may involve hypoxia-induced reflex redistribution of cardiac output with preferential shunting of blood away from the lung and kidney, the two major sources of fluid production in later pregnancy.[21, 22]

We postulate that the low perinatal mortality recorded in this study results from the integration of observed short- and long-term indices of fetal condition with other obstetric and maternal data in formulating a management plan and timing of delivery. This approach coupled with detailed screening for detectable congenital anomalies now permits more accurate assessment of fetal risk and therefore more rational planning of pregnancy management. We stress that it is not possible to base management on the score alone and that score components together with the clinical circumstances must be considered. Thus, for example, we have recommended delivery in the postdate patient with oligohydramnios even when the other variables of the profile were normal.

In summary, these data suggest that fetal risk assessment based on fetal biophysical profile scoring is an accurate method for identification of the fetus at risk for lethal asphyxia in utero. This improved accuracy combined with ease of clinical application and antepartum detection of major anomalies makes this method an attractive alternative of antepartum fetal surveillance. Fetal biophysical profile scoring rests on the principle that the more complete the examination of the fetus, its activities, and its environment, the more accurate may be the differentiation of fetal health from disease states. It seems likely that further expansion of the principle with inclusion of more fetal variables will improve the accuracy even further.

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Evaluation of a new tampon device for cytologic autocollection and mass screening of cervical cancer and its precursors

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A new autocollection tampon device for obtaining material for cervical cytologic testing was compared with cervical scraping and endocervical aspiration in order to evaluate the cytodiagnostic results. In 1428 cases there proved to be a 94% correlation between these methods. In addition, both smears were compared in a group of patients known to have neoplastic disease of the cervix. In evaluating cervical cancer both techniques detected 100% of the cases. However, the smears, both regular and tampon, were able to diagnose only 96.5% and 89% of grade 3 lesions and 86% and 81% of grades 1 and 2 lesions, respectively. False negative results occurred in 8.2% of the regular smears and 15% of the tampon smear group. These preliminary data, if confirmed by more broad and in-depth studies, suggest the new autocollection tampon may be a useful tool for mass cervical screening. (AM J OBSTET GYNECOL 1985;151:351-5.)

Key words: Cytologic autocollection device, cervical cancer, cervical biopsy, Papanicolaou smear

Since 1943, when Papanicolaou and Traut[1] first introduced cytologic testing for the mass screening of cervical carcinoma, there has been a steady decrease in the mortality from this disease. Death rates over this time have dropped from approximately 20 to 7 per 100,000.[2] Indeed, it has been shown that the death rate from cervical cancer is inversely proportional to the extent of cytologic screening in a given population[3] and that if all women were screened regularly the mortality from this disease would be virtually eliminated. However, in the female population at highest risk the annual rate of cytologic screening varies from 5% to 25%, and it is estimated that at least 10% of women in the United States have never been screened.[4] It is uncertain whether this is because of the inaccessibility of medical care, reluctance on the part of the patient to be examined, or economic factors. One solution to this problem would be a simple, self-administered, inexpensive test which could be performed, at regular intervals, by the patient at home and then mailed to a central laboratory for evaluation. In an attempt to solve the need for a mass cytologic sampling tool two methods have been evaluated with some success: vaginal irrigation and vaginal tampon devices.

In 1962, Davis[5] devised a self-irrigating method for mass screening of the cervix. His results approached those obtained by the standard Papanicolaou smear in detecting cervical abnormalities. Since that time other authors have reported on the use of the irrigation technique, some with similar[6-8] and others with divergent[9, 10] results. The use of the vaginal tampon as a collection technique was first reported by Papanicolaou[11] in 1954. Other investigators, refining this technique, also showed promising results.[12-14] The present study was undertaken to evaluate a new tampon device and its ability to act as a mass screening tool for the detection of cytologic abnormality in patients with cervical cancer and its precursors.

Material and methods

The data presented in this study are derived from three sources: (1) a new autoadministered tampon-obtained cytologic smear, (2) routine physician-obtained cervical scrapings, with endocervical aspiration (herein referred to as direct Papanicolaou smear), and (3) colposcopically directed cervical biopsies. In order to ensure objectivity of the results, both the tampon and direct Papanicolaou smear cytologic results were handled in a blinded manner with a coded number assigned to each separate specimen. Between April, 1982, and September, 1983, 1428 patients were admitted to this study. The patients came from three distinct populations. The first was a young heterogeneous social and racial group, 80% of whom were in their first trimester of pregnancy, who were seen at the Midtown Hospital in Atlanta, Georgia (group 1). The second was composed of patients being seen in a private gynecologist's office, the majority of whom were Caucasian and middle to upper class (group 2). The final group (group 3) was obtained from the Atlanta Gynecologic Oncology Center at the Georgia Baptist Medical Center, Atlanta, Georgia. The patients in group 3, both private and

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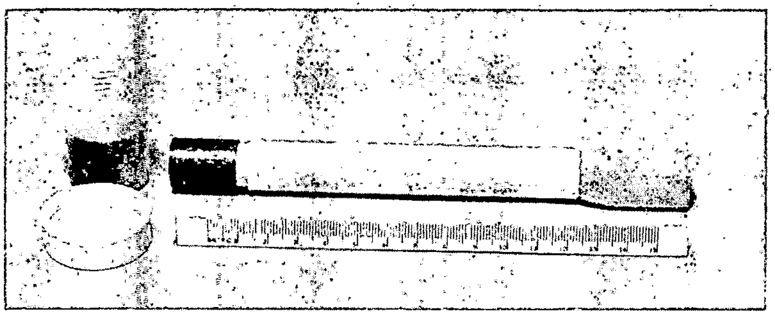

Fig. 1. The new autocollection tampon for cervical cytologic testing.

Table I. Distribution of patients in the study

Group	*No.*	*%*
1	1040	73
2	243	17
3	145	10
Total	1428	100

Table II. Tissue diagnosis in 145 cases with abnormal Papanicolaou smears (group 3)

Histologic classification	*No.*
Cervical intraepithelial neoplasia	
None	71
Grades 1 and 2	37
Grade 3	28
Cervical cancer	8
Endometrial cancer	1

service, had all been referred for colposcopic evaluation after having had previous abnormal Papanicolaou smears (Table I). The average age of the entire population studied was 27 with a range of 14 to 63 years. Approximately 56% were Caucasian and 21% were indigent.

The new autocollecting instrument was developed by Health Services Associates* (Fig. 1). Although this tampon device is similar to one designed by Kato there are distinct differences.[14] These differences mainly involve the configuration and composition of the sponge head. The new sponge is slightly larger and square in shape, thus increasing the surface area and scraping ability of the device. After several unsuccessful attempts at finding the right composition of the sponge head, a microporous polyurethane compound was selected. This new head, because of its microporous configuration, does not allow the cells to be drawn along with the fixative, into the center of the sponge. This, it was felt, would increase the number of scraped and exfoliated cells remaining on the surface of the sponge and thereby make them available for preparation and later evaluation.

After informed consent was obtained, each patient was given the tampon device along with written instructions. All consenting patients, who were not menstruating at the time of the examination, were accepted into the study. Bleeding from any other cause was not considered a reason for exclusion. The patients were encouraged to follow the supplied instructions, but no other assistance was given in obtaining the tampon cytologic specimen. The instructions directed each patient to insert the applicator and tampon into the vagina as far as it would comfortably go. The patient was then asked to move the applicator from side to side and top to bottom and finally rotate it one complete turn before removing the device. Only a small number of patients (0.5%) had trouble following the instructions and needed only to be told to read them again in order to satisfactorily complete the procedure. The patient was then instructed to separate the tampon sponge head from the applicator and place it tip down in a small vial containing 3 ml of 70% ethanol which was included in the tampon kit. Bleeding after the use of the tampon was infrequent (1.9%) and minimal. Only one patient, later found to have extensive exophytic carcinoma of the cervix, bled moderately, needing direct pressure to stop the bleeding. A physician-performed direct Papanicolaou smear was then obtained from all patients in the study. The tampon's sponge head and the direct Papanicolaou smear were sent to J. M. Webber, M.D.P.C. Laboratory, Columbus, Georgia, for preparation and evaluation. All of the direct Papanicolaou smears were processed by standard Papanicolaou methodology with the use of Harris hematoxylin nuclear stain and OG-6 and EA 36 cytoplasmic stain.[15]

Originally it was found that the cells from the tampon sponges washed off the slides during preparation. This loss of adhesiveness of the cells and failure to remain on the slides was resolved by using slides prepared by application of 20% albumin in glycerin. These slides were air dried prior to the preparation of the smear. The sponge head was carefully removed from the vial, taking care not to depress or squeeze the sponge, thus avoiding the loss of fixative containing cells in suspen-

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*Patent pending. Not yet available commercially.

Tampon Smear Class

Direct Smear Class	Total	I	II	III	IV	Unsatisfactory
Total	1428	1240	124	49	9	6
I	1231	1208	15	3		5
II	119	16	95	7		1
III	58	4	14	38 Ca× 1	2 Ca× 1	
IV	8			1 Ca× 1	7 Ca× 7	
Unsatisfactory	12	12				

Fig. 2. Comparison of cases detected by tampon and direct Papanicolaou smears.

Table III. Diagnoses of eight cases of cervical cancer by direct and tampon smears

	Direct smear			*Tampon smear*		
Cytologic diagnosis	*No.*	*%*	*False negative rate (%)*	*No.*	*%*	*False negative rate (%)*
Positive	7		0	7		0
Suspicious	1			1		
Atypical	0			1		
Total	8	100		8	100	
Negative	0			0		
Unsatisfactory	0			0		

sion. The sponge was then drawn lightly across the prepared slide. Pressure was limited to only that needed to produce a visible film of fixative on the slide surface. The slides were allowed to air dry and were then stained by the same method as that used for the direct smears. It should be noted that special precautions were necessary in order to prevent loss of cells by vigorous manipulation of the slides while making the required dips in the sequence of staining vessels.

Very gentle oscillations of <1.0 cm in amplitude achieved satisfactory staining without loss of cells. Once the technique had been perfected, preparation and staining of the tampon-obtained smears did not take a significantly longer time than the direct Papanicolaou smears. Of the 1428 patients in whom a direct and a tampon smear were obtained, overall preservation of the specimens in shipping were excellent. Twelve direct smears arrived with the slides shattered to the extent that reconstruction of the specimen was impossible. No sponge was damaged in mailing, no vials were broken, and all lids were intact and tight. There were no instances of fixative evaporation and smears were made from every specimen. However, six sponges did not contain adequate cells for interpretation. This represents only a 0.4% rate for unsatisfactory tampon smears. All specimens, both direct and tampon-obtained, were evaluated by a pathologist and cytologic technician. Satisfactory smears with both the direct and tampon methods were obtained in more than 98% of the participating patients.

All patients in group 3, after the tampon smear and direct Papanicolaou smear were obtained, underwent colposcopic or other indicated evaluation by the gynecologic oncologist.

Results

During the time this study was performed, 1428 patients used the tampon autocollecting device and then underwent cervical scraping and endocervical aspiration. Surprisingly only 0.4% of the tampon group and 0.8% of the direct smear group had unsatisfactory smears. Fig. 2 shows the results of the comparison between the tampon and direct smears. There was complete agreement between the smears in 1348 cases (94%). In 1208 cases results of both smears were reported as negative while atypical findings were re-

Table IV. Diagnoses of 28 cases of grade 3 cervical intraepithelial neoplasia by direct and tampon smears

	Direct smear					
Cytologic diagnosis	*No.*	*%*	*False negative rate (%)*	*No.*	*%*	*False negative rate (%)*
Positive	0			0		
Suspicious	26	93		25	89	
Atypical	1	3.5		0		
Total	27	96.5		25	89	
Negative	1	3.5	3.5	3	11	11
Unsatisfactory	0	0		0	0	

Table V. Diagnoses of 37 cases of grades 1 and 2 cervical intraepithelial neoplasia direct and tampon smears

	Direct smears			Tampon smears		
Cytologic diagnosis	*No.*	*%*	*False negative rate*	*No.*	*%*	*False negative rage*
Positive	0			0		
Suspicious	30	81		28	78	
Atypical	2	5		1	3	
Total	32	86		29	81	
Negative	5	14	14	7	19	19
Unsatisfactory	0			1		

ported in 95 cases. Suspicious results were obtained in 38 cases and seven cases were positive for cervical or endometrial malignancy. It is of interest that all nine known cancers in this study were detected by the tampon and direct Papanicolaou smear as either positive or suspicious findings. Table II shows the results of the tissue diagnosis in the 145 cases evaluated for abnormal Papanicolaou smears. This represents the high-risk referred group 3. In this group, 51% had tissue-diagnosed pathologic conditions. Tables III, IV, and V compare the accuracy of the tampon and direct Papanicolaou smears in diagnosing invasive cervical cancer, grade 3 cervical intraepithelial neoplasia, and grades 1 and 2 cervical intraepithelial neoplasia. In evaluating the eight cases of cervical cancer, both smear techniques detected 100% of the invasive cancers. However, direct and tampon smears were able to diagnose only 96.5% and 89% of the grade 3 lesions and 86% and 81% of the grades 1 and 2 lesions, respectively. Atypical smears were included with the positive and suspicious smears in determining the total detection rate because they represent a possible abnormal finding that needs to be evaluated further.

Comment

It has been widely accepted that screening of the female population is the key to the virtual elimination of mortality from cervical carcinoma. However, even after 40 years of Papanicolaou smear use and the widespread publicity extolling its benefits as a screening tool, a large portion of women in the United States remains unscreened or irregularly screened. A new tampon device for cytologic autocollection was tested as a mass screening technique with the potential to reach the population not currently being evaluated. By no means is this test meant to replace the standard Papanicolaou smear or a complete gynecologic examination. In fact, one of the greatest potentials for this new device is the ability to eventually bring patients who would not normally seek medical attention into the medical mainstream. However, if this technique is to become widely used, it must be easy to administer, be aesthetically acceptable to the patients, produce a good laboratory specimen which can be easily evaluated without additional specialized training, have an accuracy approaching that of the Papanicolaou smear, and have a low false negative rate.

In our population of >1400 patients, virtually 100% of the test population were able to administer the device by themselves by following simple written instructions. The patients had no trouble using the device and found it aesthetically acceptable, causing no pain or discomfort. In addition, the patients found no difficulty in preparing and mailing the tampon specimens to the receiving laboratory.

The processing of these smears was virtually identical to that currently being used for preparation of routine Papanicolaou smears. Moreover, no additional training was needed by the laboratory personnel, in either preparation or evaluation of these slides. This is a distinct advantage over the irrigation techniques previously proposed for mass screening.[5, 7, 9] In comparing the quality of smears from the direct and tampon methods, an interesting finding was noted. Occasionally the direct smears were covered by thick inflammatory exudates, making them difficult to interpret. However, the tampon smear from the same patient, probably because of a loss of adhesiveness between the cells caused by the fixative, showed an even monolayer of both epithelial and exudative cells, thus permitting easy evaluation despite the heavy inflammatory cell content. In addition, the diversity of cells (superficial, intermediate, and basal) was readily comparable to that of the direct smear and permitted assessment of any variation of cell type.

There was a favorable correlation between the tampon and direct smears. In the detection of invasive cervical cancer both methods satisfactorily detected 100% of the cases. However, in cases of grade 3 and of grades 1 and 2 cervical intraepithelial neoplasia the accuracy rates for the direct Papanicolaou smear were 96% and 86% compared with 89% and 81% for the tampon method. During this study one case of endometrial cancer was found. Both the direct and tampon methods were able to detect this cancer. Scott et al.[16] using the Draghi tampon device found a higher detection rate for endometrial cancer with the tampon method. They ascribed this to the fact that the tampon method had a greater efficacy in picking up cells from the posterior vaginal fornix, the place where the shedding endometrial tumor cells would most likely be found. However, because of the single endometrial cancer in this study, no comment as to the ability of the new tampon to diagnose this disease can be made.

False negative results occurred in six cases (8.2%) of the direct smear and 11 cases (15%) of the tampon smear. The sources of error in these cases were cytologic sampling in four of six direct smears and eight of 11 tampon smears with error in interpretation making up the remainder of the cases. These results are within the accepted range for Papanicolaou smears in which the false negative rate can range between 6% and 28%.[7]

The accuracy of any screening technique becomes similar, approaching 100%, when repeated at regular intervals. Achieving this goal necessitates a simple, widely accepted, easily evaluated, reliable mass screening tool, which this new autocollection tampon method represents.

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Combined morphologic and cytochemical grading of serous ovarian tumors

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The potentiality of DNA analysis to complement morphologic evaluation in classifying serous ovarian tumors as adenoma, borderline malignancy, or invasive adenocarcinoma was investigated in a series of 54 tumors. The DNA analyses were performed on histologic tumor sections. The primary diagnoses were borderline tumor in 24 cases and invasive adenocarcinoma in 30 (World Health Organization classification). When the specimens were reviewed, 17 of the 54 tumors were reclassified, after which the series consisted of 9 adenomas, 24 borderline tumors, and 21 invasive adenocarcinomas. Rising histologic malignancy grade was associated with increasing numbers of cells showing high DNA content. The DNA levels in the adenomas thus were within the diploid range of a normal cell population. They were somewhat higher in the borderline tumors and were highest in the invasive adenocarcinomas. Though no clear-cut intergroup demarcation was discernible, there was a subgroup of adenocarcinomas with greatly elevated DNA levels, indicating high biologic malignancy. The observations suggested that DNA analyses can complement histologic malignancy grading and can be useful for the recognition of highly malignant tumors amoung invasive adenocarcinomas. (AM J OBSTET GYNECOL 1985;151:356-61.)

Key words: Serous ovarian tumors, morphology, DNA

Despite modern methods of treatment for ovarian cancer, the mortality from this disease remains high. The prognosis may differ widely from case to case and mainly depends on the histologic tumor pattern. Thus tumors fulfilling the World Health Organization criteria for borderline malignancy[1] carry an appreciably better prognosis than do invasive adenocarcinomas.[2] Some studies have demonstrated 91% to 93% survival in the borderline cases, but only 34% in invasive adenocarcinoma.[3, 4] The histologic diagnosis therefore is important for judging the malignancy potential.

According to recommendations from the International Federation of Gynecology and Obstetrics[5] and the World Health Organization,[1] the histologic appearance of the primary ovarian tumor should provide the basis for classification and malignancy grading. Though the importance of universally similar, or preferably identical, criteria for histologic tumor diagnosis is clear, earlier studies of various tumor types[6-8] have shown that different pathologists may disagree in their interpretation of the same sections. A more objective method for correct malignancy grading of tumors is therefore highly desirable.

As early as 1966, Taylor[9] demonstrated that the levels of DNA in adenocarcinoma of the ovary were related to the histologic grade of malignancy. Increasing aneuploidy (nondiploidy) thus was seen to accompany increasing degree of malignancy. A recent study has confirmed these findings.[10]

In the present study, DNA levels in serous ovarian tumors were compared with histologic assessment of the same tumors. The aim was to evaluate the usefulness of DNA analysis as an objective and accordingly reproducible technique in assisting World Health Organization grouping as adenoma, borderline malignancy, or invasive carcinoma. The DNA analyses were made on sectioned tumor specimens, which permitted direct comparison between the histologic evaluation and the DNA measurements in the tumor cells.

Material and methods

Patients. The patients had been referred to the Radiumhemmet for postoperative treatment of serous malignant or borderline ovarian tumors during the period 1975 to 1980. The International Federation of Gynecology and Obstetrics clinical stage of the tumors was IA-C or IIA.[5] The primary surgical treatment was performed at various hospitals in the Stockholm region, where the tumors had been histologically classified and graded.

The histologic specimens were reviewed at the Karolinska Hospital by an experienced pathologist (C. S.), who was unaware of the earlier diagnosis and of the

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patients' clinical fate. This review excluded from the series tumors which did not fulfill the World Health Organization criteria for serous ovarian tumor. The 54 remaining tumors were thereafter grouped according to World Health Organization[1] and International Federation of Gynecology and Obstetrics[5] classification as serous adenoma (IA), serous borderline tumor (IB) or invasive adenocarcinoma (IC). The adenocarcinomas were then graded by malignancy, with guidance from the degree of cellular pleomorphism, nuclear atypia, mitosis frequency, and solid growth, as highly, moderately, or poorly differentiated tumors[11] (Table I).

During the follow-up period (mean, 5 years; range, 2.5 to 8 years), eight patients died because of the ovarian tumor. All deaths occurred within 2.5 years of the primary diagnosis.

Histological material. Paraffin-embedded tissue resected at the primary operation was used in the analyses. A tumor-representative block was selected in each case. Two consecutive sections of 4 µm thickness were cut from each block. After deparaffining, one section was stained with hematoxylin-eosin solution. The other was stained according to the Feulgen technique (acid hydrolysis with 5N hydrochloric acid, 22° C, 60 minutes) after refixation in 10% neutral buffered formalin.[12]

With guidance from the hematoxylin-eosin–stained sections, representative areas in the Feulgen preparations were selected for photography. Usually 10 fields were photographed in each case. The fields were chosen from different areas where the tumor tissue was well preserved histologically and where the architecture of the tumor tissue allowed DNA analysis. The general goal was to select representative fields throughout the entire tissue section. The selection was done by an experienced pathologist (K. E.), without foreknowledge of the previous diagnosis in the case or of the clinical course. A Leitz photomicroscope was used, with 40×/1.0 oil objective (refractory index, 1.518) in monochromatic light and wavelength of 546 nm. The sensitivity of the film (Kodak Technical Pan 2415) was 29 DIN and the developing time was 4 minutes at 22° C (Kodak D_{19}).

DNA measurements. On the developed films the DNA content of individual cells was measured. The photographic cytophotometric method used was a modification of that described by Adams[13] in 1968. The method is based on light transmission from the Feulgen-stained nuclei, the blackness of which is taken as a measure of the DNA content. Comparative studies between this method and conventional rapid-scanning microspectrophotometry[14] showed close conformity.

Table I. Grading of ovarian tumors in study

Case No.	*Age (yr)*	*Histologic classification*		*Cells >2.5c (%)*
		Original	*Revised**	
1	74	IB	IA	25
2	74	IB	IA	44
3	72	IB	IA	22
4	69	IB	IA	32
5	69	IB	IA	42
6	63	IB	IA	20
7	56	IB	IA	25
8	58	IB	IA	36
9	51	IC	IA	43
10	75	IB	IB	65
11	73	IB	IB	42
12	60	IB	IB	50
13	69	IC	IB	75
14	69	IB	IB	67
15	62	IB	IB	66
16	60	IB	IB	47
17	59	IB	IB	56
18	52	IC	IB	56
19	51	IB	IB	55
20	53	IB	IB	17
21	53	IC	IB	37
22	50	IB	IB	47
23	51	IC	IB	72
24	49	IB	IB	39
25	46	IB	IB	48
26	46	IC	IB	21
27	43	IB	IB	60
28	42	IB	IB	29
29	40	IB	IB	59
30	38	IC	IB	44
31	34	IC	IB	62
32	37	IB	IB	29
33	32	IC	IB	37
34	41	IC	IC (h)	56
35	40	IC	IC (h)	26
36	40	IC	IC (h)	53
37	40	IC	IC (h)	57
38	74	IC	IC (m)	28
39	54	IC	IC (m)	81
40	62	IC	IC (m)	63
41	58	IC	IC (m)	94
42	75	IC	IC (m)	99
43	63	IC	IC (m)	90
44	55	IC	IC (l)	93
45	55	IC	IC (l)	77
46	60	IC	IC (l)	98
47	70	IC	IC (l)	69
48	67	IC	IC (l)	96
49	55	IC	IC (l)	96
50	64	IC	IC (l)	99
51	60	IC	IC (l)	92
52	55	IC	IC (l)	82
53	54	IC	IC (l)	97
54	50	IC	IC (l)	95

*Differentiation represented by h, high; m, moderate; l, low.

In each histologic slide 100 tumor cells were analyzed for DNA content. Within the photographed tumor areas individual tumor cells were selected by means of morphologic criteria. All tumor cells that could be analyzed (e.g., no overlapping of the Feulgen-stained nuclei), were measured at random. To define the normal diploid (2c) value, approximately 50 control cells—fibroblasts and/or endothelial cells—were similarly analyzed in normal tissue adjoining the tumor. To permit

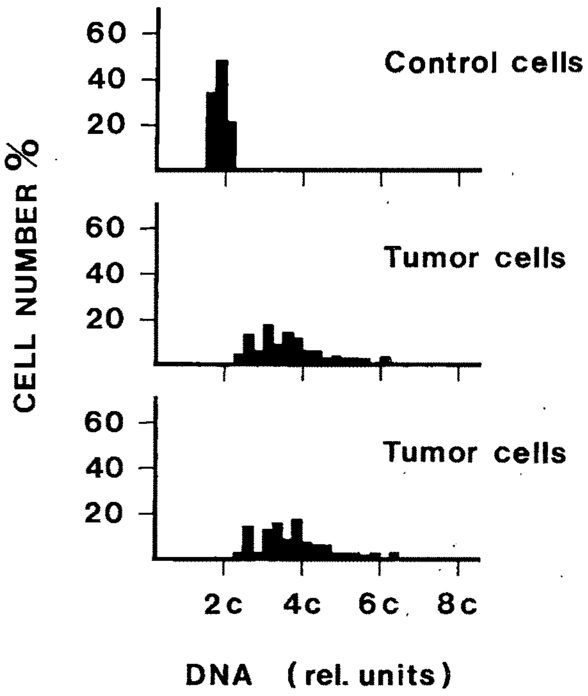

Fig. 1. Example of DNA distribution patterns of normal fibroblasts and endothelial cells *(control cells)* and of tumor cells before recalculation according to the median value of the control cells *(intermediate histogram)* and after recalculation *(lower histogram)*.

comparisons between the DNA levels in different tumors, DNA content was expressed in c units, 2c being defined from the median value (P50) found in the control cells. An upper limit of 2.5c was set for diploid cell populations, so as to distinguish them from nondiploid types. This limit was empirical, based on the observation that it is not exceeded by most (>90%) of the control cells. The percentage of tumor cells with DNA >2.5c was taken as a measure of nondiploidy. Fig. 1 illustrates an example of how the DNA values from a tumor were recalculated according to the internal standard. The upper histogram shows the distribution pattern of the control cells, the intermediate that of the uncorrected tumor cell population, and the lower that of the profile after correction.

Results

The 54 cases remaining in the series after histologic review of the original specimens are surveyed in Table I. Initially 24 of the tumors were classified as of borderline malignancy (IB) and 30 as invasive adenocarcinoma (IC). The revised classification was adenoma (IA) in 9 cases, borderline tumor (IB) in 24 and invasive adenocarcinma (IC) in 21 cases. Of the nine adenomas, eight had previously been classified as borderline tumors and one as adenocarcinoma. Eight tumors initially judged to be adenocarcinomas were reclassified as borderline malignancies.

The DNA patterns are exemplified in Fig. 2. The tumors which at review of the histologic specimens were classified as adenoma (IA) showed DNA values around diploid level. In the borderline tumors (IB) the values were slightly higher but still within the DNA limits in normal proliferating cells. Among the invasive carcinomas (IC) there were some with DNA patterns resembling those of borderline tumors, while others were composed of cells with very high and scattered DNA contents which significantly exceeded the values in normal proliferating cells. At the histologic review, most of the invasive adenocarcinomas with DNA pattern resembling borderline malignancy were graded as highly differentiated, whereas the invasive carcinomas with DNA content indicating severe aneuploidy were graded as poorly or moderately differentiated.

For each of the 54 tumors in the series, the proportion of cells with DNA content exeeding 2.5c was calculated as a measure of nondiploidy (Table I). The association between the primary diagnosis and nondiploidy is illustrated in Fig. 3 and that between the review diagnosis and nondiploidy in Fig. 4. The distribution of malignancy grades—high, moderate, or poor differentiation—within group IC is also shown in Fig. 4.

Tumor populations with comparable degrees of nondiploidy were found in the groups primarily classified as IB or IC (Fig. 3). The latter group, however, also contained tumors with very high proportions (>80%) of nondiploid cells. A similar relationship between tumor DNA content and histologic evaluation was found when the classification was revised (Fig. 4), i.e., lowest DNA levels in adenomas (IA) and highest in adenocarcinomas (IC). Among the invasive adenocarcinomas, those of moderate or poor differentiation showed the highest DNA values, whereas the DNA content in the highly differentiated tumors did not essentially differ from the values in the borderline group (IB).

In all of the eight patients who died of ovarian malignancy, both the primary and the reviewed morphologic diagnosis were IC. At review of the specimens the differentiation grade was found to be moderate or poor. In all eight tumors there was severe nondiploidy, with more than 80% nondiploid cells (Figs. 3 and 4).

Comment

In recent years many new treatment modalities have been introduced for malignant ovarian tumors, particularly as a result of progress in radiotherapy and chemotherapy. Several prospective randomized studies have been undertaken to evaluate such regimens. As in earlier studies, the prognosis has been shown to be determined by a variety of factors, including type of tumor, clinical stage and the histologic grade of malignancy. Recently a multivariate analysis[15] demonstrated the histologic malignancy grade to be well correlated to survival within the clinical stages IA-C and IIA.

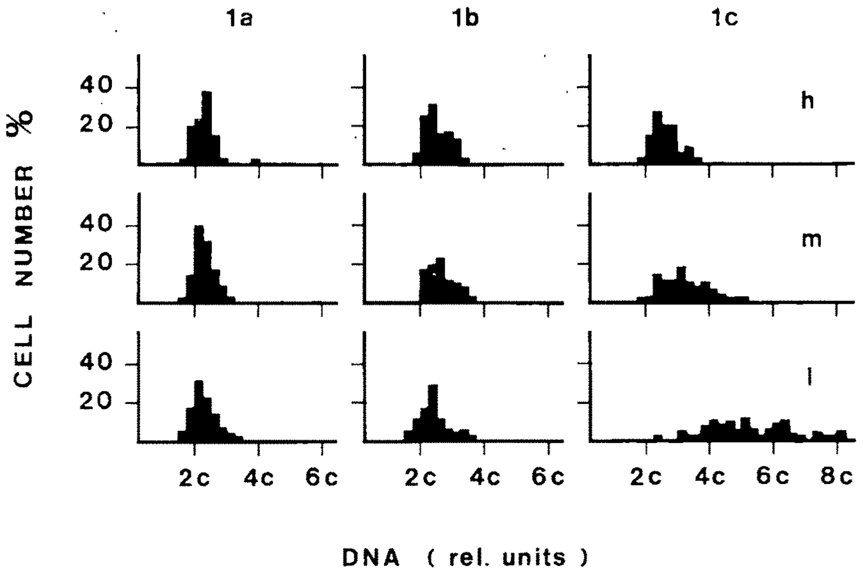

Fig. 2. Examples of DNA distribution patterns in serous ovarian tumors with morphologic diagnosis of adenoma *(IA)*, borderline tumor *(IB)* or invasive adenocarcinoma *(IC)*. The adenocarcinomas are subdivided according to tumor differentiation: *h*, high; *m*, moderate: *l*, low.

For comparability of treatment results, both within single randomized series and between series at different treatment centers, homogeneity of histologic classification and of malignancy grading are decisive. For the individual patient, too, such malignancy grading is of great importance. The high risk of side effects from currently employed chemotherapy underlines the need for standardized malignancy grading as a basis for choice of postoperative management.

Great efforts have therefore been made to define reproducible histologic criteria and to disseminate them among pathologists, e.g., by the World Health Organization.[1] Despite all attempts to achieve objectivity, however, histologic diagnosis based on light microscopy remains a subjective assessment that is influenced by the examiner's personal experience in the field. Although this influence is of minor significance in regard to tumors of typical appearance, diagnostic routines frequently must deal also with tumors that cannot readily be assigned to the groups described in manuals.

Measurement of DNA content, with standardized procedure, is an objective and hence reproducible method. In a recent study of serous ovarian tumors there was a close relationship between the nuclear DNA content and the malignancy potential of the tumors. The biologically highly malignant tumors were identifiable with surprisingly good accuracy.

The question posed for the present study was whether DNA analysis could also provide a basis for grouping of serous ovarian tumors as adenoma, borderline malignancy, or adenocarcinoma. We therefore compared the DNA levels with the histologic evaluation. To make the comparison as direct as possible, the DNA analyses were performed on sections from the tissue specimens that had been used for the histologic examinations. The study comprised 54 tumors primarily diagnosed as serous borderline malignancy or serous invasive adenocarcinoma. Review of the specimens led to revision of the diagnosis in 17 (31%) of the series, a figure which agrees well with previous reports.[6-8]

The distribution pattern of DNA (Fig. 2) showed that in the tumors reclassified as adenoma (IA) the values mostly were within the limit (<2.5c) for a normal diploid cell population. Between adenoma and borderline tumor, however, there was no clear demarcation in regard to DNA pattern. The borderline tumors thus included some that were comparable with adenoma in this respect and some with fairly abundant cells showing DNA levels of >2.5c. These latter nevertheless did not exceed the tetraploid (4c) values that can be seen in normal prolife-rating cell populations. Because of the imprecise transition in DNA values from adenoma to borderline malignancy, the method does not permit definite classification of a tumor as benign (IA) or of borderline type (IB), implying risk of subsequent metastasis and death from the disease. The same demarcation is also recognized as being difficult in histologic evaluation.

Among the invasive adenocarcinomas there were some with DNA distribution curves indistinguishable from those of borderline tumors, but there also was a group with strongly aneuploid cell populations, containing cells with very high and scattered amounts of DNA. At the histologic review all of these aneuploid tumors were graded as adenocarcinoma of moderate

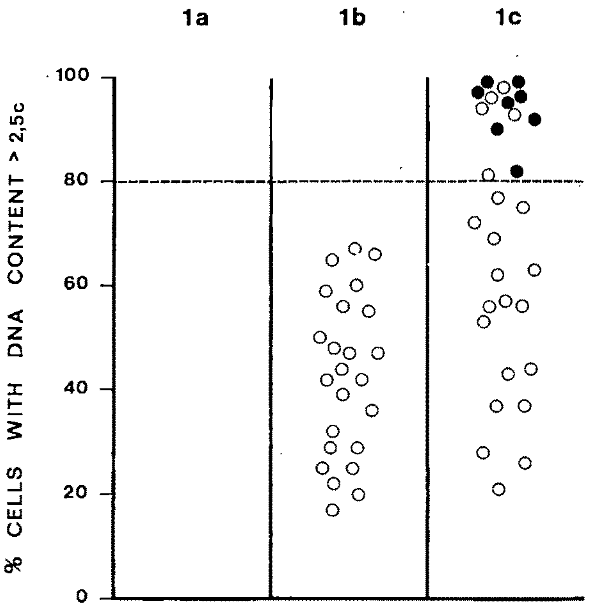

Fig. 3. Percentage of cells with DNA content >2.5c of normal control cells in tumors orginally classified as borderline malignancy *(IB)* or invasive adenocarcinoma *(IC)*. *Open circles,* survivors; *solid circles,* deaths because of tumor.

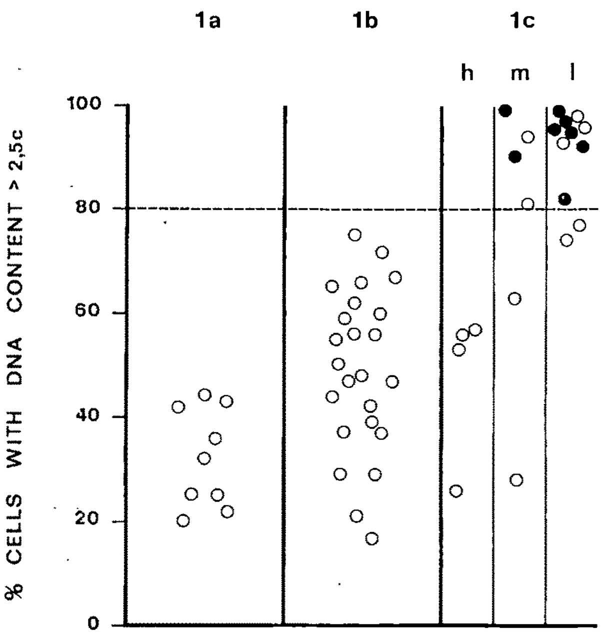

Fig. 4. Percentage of cells with DNA content >2.5c of normal control cells in tumors reclassified as adenoma *(IA)*, borderline malignancy *(IB)*, or invasive adenocarcinoma *(IC)*. The adenocarcinomas are subdivided according to tumor differentiation: *h*, high; *m*, moderate; *l*, low.

or poor differentiation, and all of the deaths in the follow-up period occurred in this group.

The tumors judged at review to be highly differentiated invasive adenocarcinoma showed DNA distribution curves of the type found in borderline tumors. The clinical behavior of these adenocarcinomas likewise resembled that of the borderline tumors in that no patient died because of the tumor. Although the number of cases was too small to permit firm conclusions, the results indicate the possibility that serous invasive cancer showing DNA content within the limits for normal tissues can be managed in the same way as borderline tumors.

Compared with histologic assessment, analysis of DNA content by means of standardized procedure is objective and accordingly a more reproducible method. DNA analysis, however, does not always permit differentiation between normal tissue and tumor tissue. Many tumors are composed of entirely diploid cell populations, and the DNA curves therefore are identical with those of normal tissue. The finding of a diploid cell population in a tumor indicates only a slow growth rate and by no means precludes metastasis.[15] Conventional histologic examination thus remains decisive for the distinction between adenoma and borderline tumor. The present study suggests that, despite the subjective factor in histologic malignancy grading, its reliability in this distinction is relatively high.

The histologic demarcation between tumors of borderline malignancy and true invasive adenocarcinoma is likewise subjectively influenced. Many tumors histologically classified as invasive cancer are of low malignancy, which is reflected in patient survival times comparable with those for borderline tumor. The main importance of DNA analysis in ovarian tumors is its high specificity in distinguishing between high and low degrees of malignancy. DNA analysis, as an objective technique, thus constitutes a useful diagnostic complement, partly as an aid in histologically doubtful cases, but above all for identification of the highly malignant tumor forms. A combination of histologic evaluation and DNA analysis therefore appears to offer prospects of heightened diagnostic accuracy in serous ovarian tumors.

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The effect of a prostaglandin synthetase inhibitor on the hormonal profile and the endometrium in women

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A prostaglandin synthetase inhibitor, naproxen, was given continuously throughout the menstrual cycle at a dose of 250 mg twice daily to 10 healthy fertile women (group 1) and at a dose of 1000 mg per day to eight women in the secretory phase (group 2). Blood samples were withdrawn three times a week during a control cycle and during the treatment cycle. Luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol, and progesterone were analyzed. Endometrial biopsies were taken in the secretory phase of the control cycle and in the treatment cycle of group 1 and on the first day of menstruation of group 2. Naproxen treatment did not suppress ovulation in any cycle and did not affect the corpus luteum function either in group 1 or in group 2. In only one of the endometrial samples taken in the secretory phase (group 1) was the density of the lysosomes increased in the treatment cycle compared to the control cycle. The specimen taken during the early menstrual period (group 2) showed an increase in glandular epithelium and height following naproxen treatment. Furthermore, a significant increase in the number of plasmolemmal vesicles per square micrometer was observed in the capillary endothelial cells after the administration of 1000 mg of naproxen per day. This suggests that the transcellular exchange of water-soluble molecules in the endothelial cells was more active after the administration of a prostaglandin synthetase inhibitor than in the control group. In spite of the significant morphologic changes observed in the naproxen-treated material the onset of menstrual bleeding could not be prevented. The mechanism of the onset of menstruation needs to be further investigated. (AM J OBSTET GYNECOL 1985;151:361-8.)

Key words: Endometrium, hormones, prostaglandin synthetase inhibitor

Animal studies have suggested that prostaglandins are mediators of ovulation as well as luteolysis.[1, 2] A similar function of prostaglandins in women has not yet been convincingly demonstrated. However, in vitro studies by Hamberger et al.[3, 4] indicate that prostaglandin $F_{2\alpha}$ in the presence of norepinephrine may induce luteolysis in human corpora lutea in vitro as well as in vivo.

Administration of prostaglandin synthetase inhibitors to a subhuman primate has been demonstrated to interfere with rupture of the follicular membrane without affecting luteinization, leading to the "blocked

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Table I. Characteristics of control and treatment cycles in 10 women treated with 500 mg of naproxen daily (Group 1)

	Control				*Treatment*			
Subject No.	*Cycle length (days)*	*Days of luteinizing hormone peak**	*Length of secretory phase (days)*	*Ovulation†*	*Cycle length (days)*	*Days of luteinizing hormone peak**	*Length of secretory phase (days)*	*Ovulation†*
1	28	14	14	Yes	27	14	13	Yes
2	26	17	9	Yes	27	17	10	Yes
3	25	9	16	Yes	25	8	17	Yes
4	27	13	14	Yes	28	12	16	Yes
5	28	9	19	Yes	30	18	12	Yes
6	28	16	12	Yes	30	19	11	Yes
7	35	22	13	Yes	28	17	11	Yes
8	35	24	11	Yes	30	16	14	Yes
9	35	22	13	Yes	32	18	14	Yes
10	27	13	14	Yes	31	17	14	Yes

*Length of proliferative phase

†Progesterone levels >16 nmol/L for at least 5 days.

ovum" state. However, a previous study by Chaudhuri and Elder,[5] in 1976, using aspirin (1.8 to 3.6 gm daily) administered to human volunteers did not affect the ovulation process as evidenced by circulating steroid hormonal levels. The present study was undertaken to evaluate the possible effect of a more potent prostaglandin synthetase inhibitor, naproxen, on ovulation and luteolysis. In addition, the morphologic features of the endometrium in the secretory phase were investigated.

Material and methods

Eighteen apparently healthy women with proved fertility and a history of regular menstrual cycles of 26 to 35 days for a minimum of 3 months prior to admission were recruited for the study. None of them had used steroidal contraceptives or an intrauterine contraceptive device during the preceding 3 months and none had an abortion within the last 3 months or a delivery within a year prior to admission. Before entry into the study a complete gynecologic examination including Papanicolaou smear was carried out. The participants were instructed to use only barrier methods for contraception during the study period.

Following approval of the Ethical Committee of the Karolinska Hospital, a prostaglandin synthetase inhibitor (naproxen, Astra Syntex, Södertälje, Sweden) was administered at a daily dose of 500 mg to 10 of the women during one complete menstrual cycle (group 1). Eight other women received 1000 mg of naproxen daily from day 8 after the estimated time of ovulation (determined by basal body temperature) to the onset of menses (group 2).

Group 1. Blood samples were withdrawn three times weekly during one control cycle and one treatment cycle. Endometrial biopsies were taken only once per cycle between cycle days 21 and 24.

Group 2. During the study basal body temperature was used to indicate the time of ovulation. During the luteal phase three blood samples were withdrawn to confirm ovulation.[6] During both the control cycle and the treatment cycle from day 12 after the estimated time of ovulation, blood samples were withdrawn every eighth hour (7:00 AM, 3:00 PM,, and 11:00 PM) until the onset of menstrual bleeding. An endometrial biopsy was taken on the first day of bleeding. The blood samples were analyzed for luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol, and progesterone by radioimmunoassay methods (kits from Biodata, Coinsins, Switzerland). The lengths of preovulatory and postovulatory phases in the control cycles were compared to those of the treatment cycles by Student's paired *t* test.

The specimens were taken with a Randall curette, without dilatation of the cervix and without anesthesia. One part of the biopsy was immediately fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4) and further processed for electron microscopy. Another part of the biopsy was fixed in Bouin's solution, embedded in paraffin, and stained in hematoxylin and eosin for light microscopic examination. The material obtained for electron microscopy was further divided into two parts. One of these was prepared for histochemical examination of acid phosphatase according to the method described by Smith and Henzl.[7] The other part was subjected to fixation in 1% osmium tetroxide dissolved in phosphate buffer (pH 7.4) and embedded in Epon epoxy resin according to the method described by Luft.[8] The sections were then mounted on unsupported 200-mesh copper grids and stained with uranyl acetate[9]

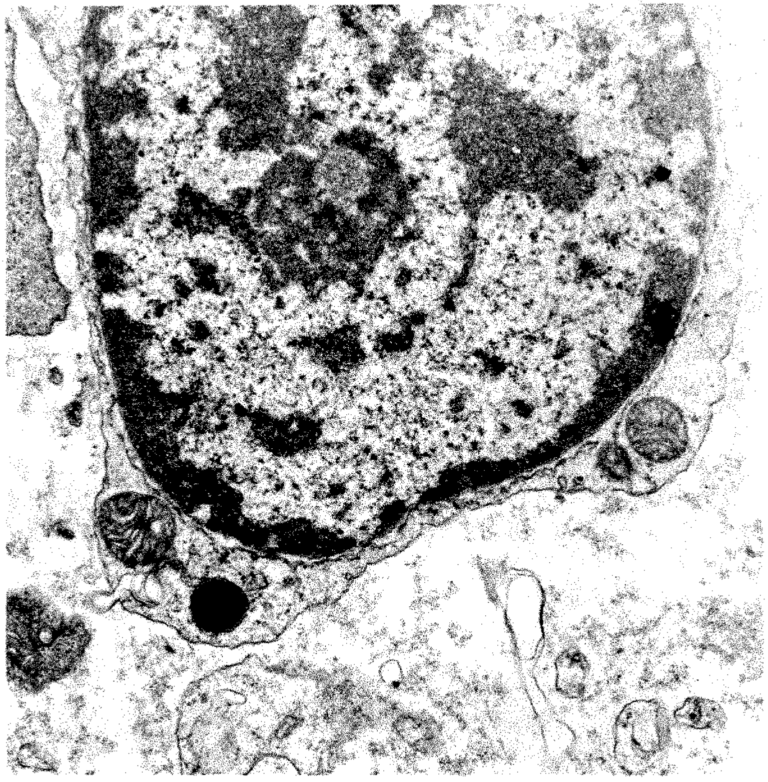

Fig. 1. Part of a cell from the endometrial stroma showing a dense lysosome with clearly distinguished membrane. The endometrium was obtained on cycle day 25 following treatment with naproxen. (Original magnification ×30,000.)

and lead citrate.[10] The specimens were examined by a Zeiss II electron microscope.

Results

All women completed the study. However, in one woman the endometrial biopsy obtained in the treatment cycle was insufficient for microscopic examination.

Group 1. Table I shows the length of the control and treatment cycles in the 10 women of group 1 as well as the length of the proliferative and secretory phases. No statistical difference was found in the length of the preovulatory and postovulatory phases when the control cycles were compared to the treatment cycles.

Naproxen treatment at a dose of 500 mg did not suppress ovulation in any cycles studied as evaluated from progesterone levels. All women fulfilled the criteria of a normal ovulatory cycle in the control month as well as in the treatment cycle (preovulatory estradiol peak of 700 pmol/L and progesterone levels above 16 nmol/L for a minimum of 5 days according to Landgren et al.[6]). Therefore, there is no evidence that the treatment with naproxen did affect the corpus luteum function. Also the prolactin levels remained low within the normal range during the naproxen treatment.

The steroid pattern observed in the treatment cycles was further supported by the endometrial histologic findings. The histologic data corresponded to the days after midcycle luteinizing hormone surge in 15 of 19 biopsies, allowing an error of ± 1 day in interpretation. In the remaining three biopsies two belonged to the control cycles and were considered to reflect the normal variation in the histologic pattern during the menstrual cycle. Only one biopsy obtained in the treatment cycle did not correspond to the criteria set for the histologic dating of the endometrium.[11] This biopsy was classified as secretory endometrium corresponding to the luteinizing hormone surge plus 11 days. The biopsy was, however, taken on the fourteenth day after the midcycle surge of luteinizing hormone, 3 days prior to the onset of menstruation.

The presence of lysosomes in the cytoplasm of the stromal cells was examined in the electron microscope by histochemical methods for the localization of acid phosphatase as well as ordinary electron microscopic methods. Specifically stained granules of acid phosphatase were found in the lysosomal bodies as well as in the endoplasmic reticulum of the cytoplasm. The number and size of the lysosomes varied markedly in the endometrial material without any significant cor-

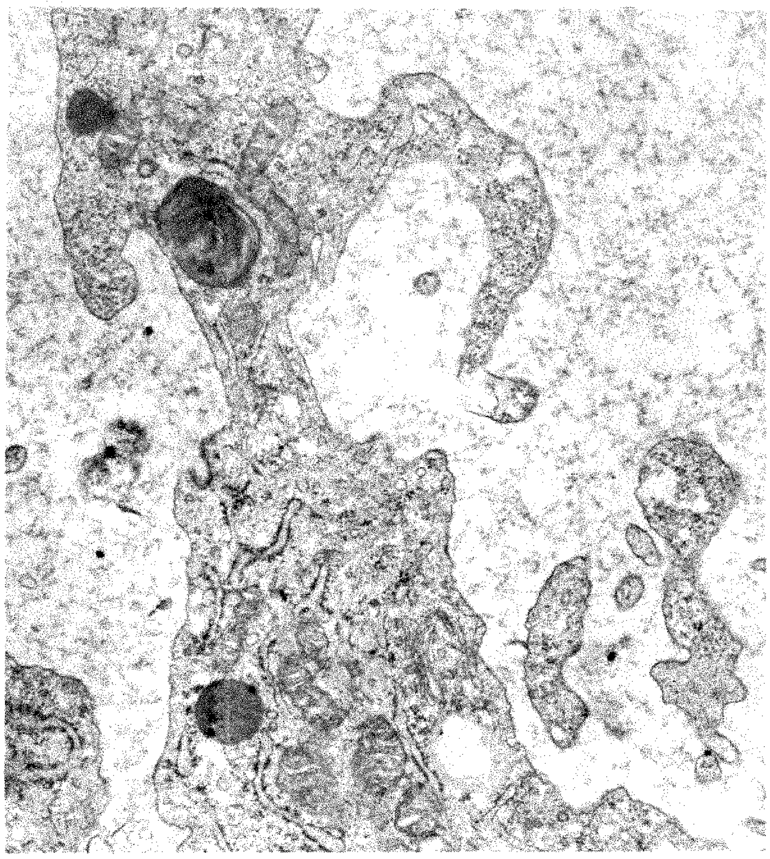

Fig. 2. Part of a cell from the endometrial stroma of material obtained on cycle day 25 of the control cycle. Note that the density of the lysosomes is less than in Fig. 1. (Original magnification ×30,000.)

Table II. Indices of endometrial activity before (control) and after 8 days of administration of 1000 mg of naproxen*

	No glands/μm²	*Glandular diameter (μm)*	*Glandular epithelial height (μm)*	*Predecidual reaction (scores)*	*Neutrophilic infiltration (scores)*
Before naproxen (control)	25.6 (19.9-32.9)	36.1 (26.1-49.9)	15.3 (13.5-17.4)	1.6 (0-2.9)	2.0 (1.2-3.5)
After naproxen	21.4† (13.2-34.7)	61.9‡ (43.9-87.3)	17.1§ (14.2-20.6)	1.9† (1.0-3.4)	1.1‡ (0-1.4)

*Geometric mean values with 95% confidence limits in parentheses.
†Not significant.
‡$p < 0.05$.
§$p < 0.02$.

relation to the treatment. In the biopsies obtained late in the secretory phase, the lysosomes often occurred in a large number in the cytoplasm and their size was slightly larger than that of those observed earlier in the postovulatory phase. In one of these cases the density of the lysosomes was increased in the endometrium obtained in the late secretory phase of the treatment cycle (Fig. 1) when compared to that in the control cycle (Fig. 2). The lysosomal membranes observed in the treatment cycle were intact with a lining clearly distinguishing the lysosomes from the surrounding cytoplasm. Whether this observation reflects an increased membrane stability due to the treatment remains to be further investigated.

Group 2. Naproxen at a dose of 1000 mg/day given during the secretory phase did not affect the corpus luteum function as reflected by luteal phase length and circulating estradiol and progesterone levels. Table II shows the endometrial activity before and after naproxen treatment.

The daily administration of 1000 mg of naproxen seemed to have a significant effect on the human endometrium in spite of the fact that the circulating hormone levels did not vary from those of the control cycle. At the time of the onset of menstruation, significantly less neutrophil infiltration ($p < 0.05$) was found in the endometrium in the treatment cycle when compared to the control cycle. The glandular epithelium was significantly less diminished ($p < 0.02$) and the diameter of the glands larger ($p < 0.05$). The histologic appearance of the endometrium after the treatment with 1000 mg of naproxen therefore rather corresponds to that of a late secretory phase than that of a premenstrual mucosa.

There seem to be some interesting findings in the morphometric assessment. The diameter of glands, glandular epithelial height, and leukocytic infiltration were significantly altered when the treatment cycle was compared to the control cycle. No effect was seen on the number of glands and predecidual reaction. Following the naproxen treatment, the histologic dating rather corresponded to a late secretory phase rather than a premenstrual phase.

As in group 1 the presence of granules representing acid phosphatase was studied in the cytoplasm of the stromal cells under the electron microscope after histochemical staining of the endometrial material. Most of the granules were attached to the membranes of the endoplasmic reticulum and no difference was found between the control and the naproxen-treated samples as to the distribution of these granules in the cytoplasm. The lysosomes had a moderate optical density and scanty granules were found inside. No difference was observed between the control and the naproxen-treated material with regard to the lysosome appearance. The endometrial samples of group 2 were all obtained at the onset of the menstrual bleeding, and the ultrastructure of the capillary endothelial cells was subjected to a detailed examination by the routine embedding and staining for transmission electron microscopy. The presence of plasmolemmal vesicles located in the periphery of the endothelial cell cytoplasm was assessed by calculating the number of vesicles per square micrometer. As illustrated in Fig. 3 the plasmolemmal vesicles appeared scantily distributed in the periphery of the cytoplasm in the material obtained from the control cycle. The geometric mean value from the eight women participating in the study was 7.2 vesicles per square micrometer (95% confidence limit, 5.1 to 10.1). On the contrary, a significantly increased number of plasmolemmal vesicles per square micrometer ($p < 0.02$) was found after the naproxen treatment. Fig. 4 shows a large number of plasmolemmal vesicles closely packed at the cytoplasmic border. The geometric mean value of the eight participating women was 13.1 vesicles per square micrometer (95% confidence limit, 9.5 to 18.0).

Comment

The results of the present study clearly indicate that the prostaglandin synthetase inhibitor naproxen does not significantly influence the normal menstrual cycle when given in therapeutic doses. In this respect our findings are in agreement with previous studies by Chaudhuri and Elder[5] who studied the effect of aspirin in therapeutic doses in women. Nevertheless, Toppozada et al.[12] suggested a possible shift of midcycle endocrine events to the left when indomethacin was given in doses of 75 mg daily from cycle day 5 to the onset of menstrual bleeding.[12] In their study the blood samples were not taken regularly throughout the cycle. Thus the time of ovulation was only approximately estimated. The results given by Toppozada et al. are therefore difficult to interpret.

The lack of influence of the prostaglandin synthetase inhibitors observed in group 2 of the present study may, however, be explained by the low dose of naproxen. Animal studies with other types of prostaglandin synthetase inhibitors, such as indomethacin, have shown a significant inhibition of ovulation when these substances are given in high doses.[13] Indomethacin given in a dose of 10 mg/kg intramuscularly daily was reported to completely block ovulation in rhesus monkeys.[13] Therefore, it cannot be excluded that doses of naproxen ten times higher may have a similar effect in humans. However, because of the toxicity of these drugs, such doses are of no clinical interest.

Administration of 75 mg of indomethacin or 500 to 1000 mg of naproxen daily 1 to 2 days prior to expected menstruation has been reported to postpone the onset of menstrual bleeding.[14] It has therefore been suggested that prostaglandin synthetase inhibitors may interfere with the timing of menstrual bleeding. In the present study no prolongation of the secretory phase could be observed in the treatment cycle when compared to the control cycle. It should, however, be emphasized that the length of the secretory phase in normally menstruating women varies between 12 and 17 days.[15] Further studies on the influence of prostaglandin synthetase inhibitors on the circulating steroid levels and the endometrial structure, when administered in the premenstrual phase, might therefore be necessary.

Although no significant difference was found as to the dating of the human endometrium in the control cycle when compared to the treatment cycle with 500 mg of naproxen daily in group 1, the results obtained with 1000 mg of naproxen daily in group 2 indicated a limited effect in the late secretory phase. The electron microscopic examination of the material revealed a slight difference as to the lysosomal structure suggesting a stabilization of the lysosomal membranes in the treatment cycle. Previous studies by Weissman

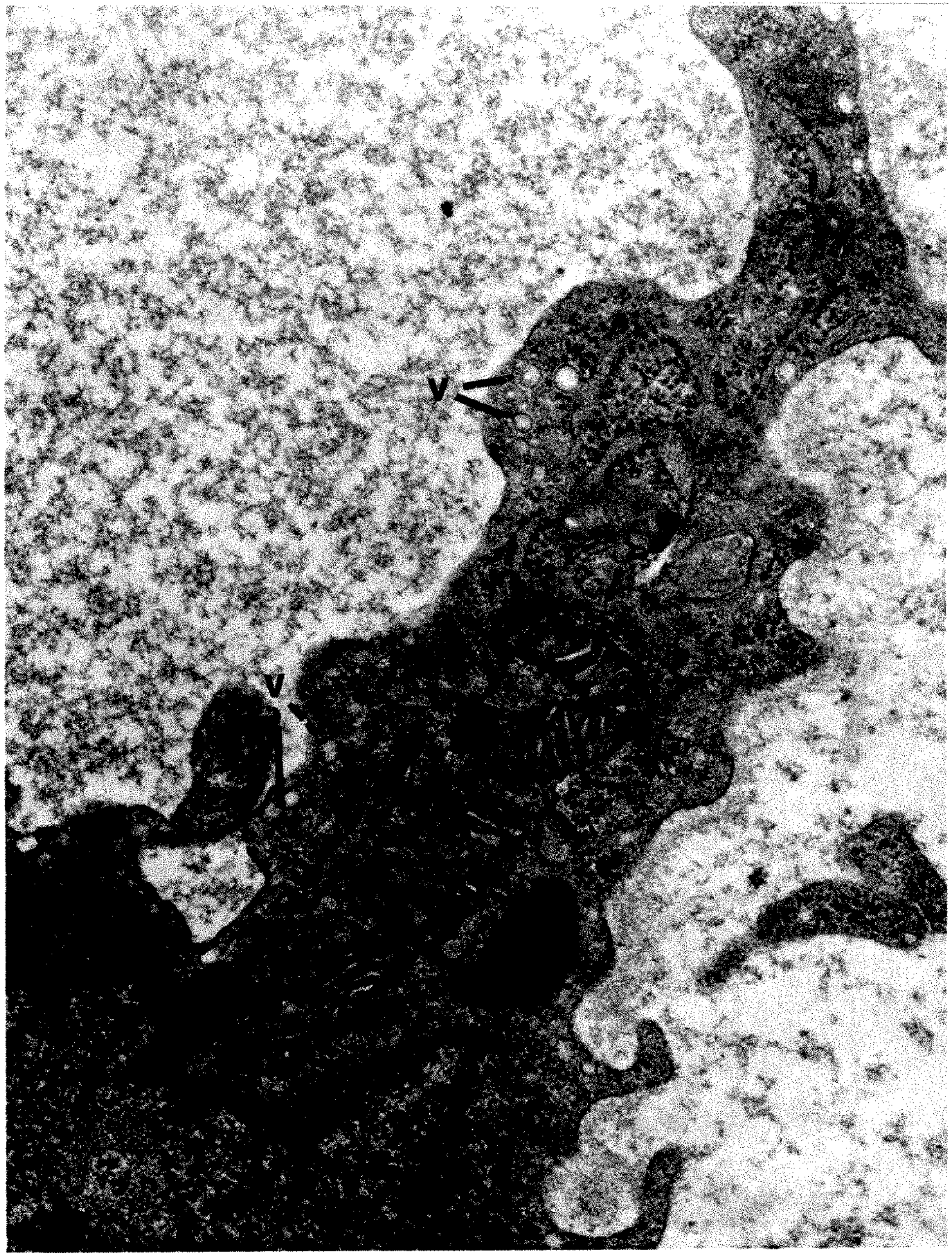

Fig. 3. Part of an endothelial cell of endometrium obtained in the control cycle on the day of onset of menstrual bleeding. The cytoplasmic matrix is dense. The lysosome *(L)* shows a moderate density. A few plasmolemmal vesicles *(v)* are scattered along the cytoplasmic border. (Original magnification ×55,400.)

et al.[16] have suggested that prostaglandins and cyclic nucleotides may be involved in lysosomal enzyme release.[16] The effect of circulating progesterone and estradiol on the lysosomal membrane stability may also be taken into consideration.[17, 18]

The significant increase in the number of plasmolemmal vesicles per square micrometer observed in the capillary endothelial cells after the naproxen treatment also seems to indicate that the transcellular exchange of water-soluble molecules is more active after naproxen treatment. In this respect it is noteworthy that mice injected intraperitoneally with a lipid-substituted derivative of cyclic adenosine monophosphate (dibutyryl cyclic adenosine monophosphate) showed a significant increase in the number of plasmolemmal vesicles per square micrometer of the endothelial cytoplasm of brain capillaries 5 and 20 minutes after the injection.[19] It is known that prostaglandin synthetase inhibitors block the formation of prostaglandins E_2 and $F_{2\alpha}$[20] and obviously interfere with the adenosine 5′-di-

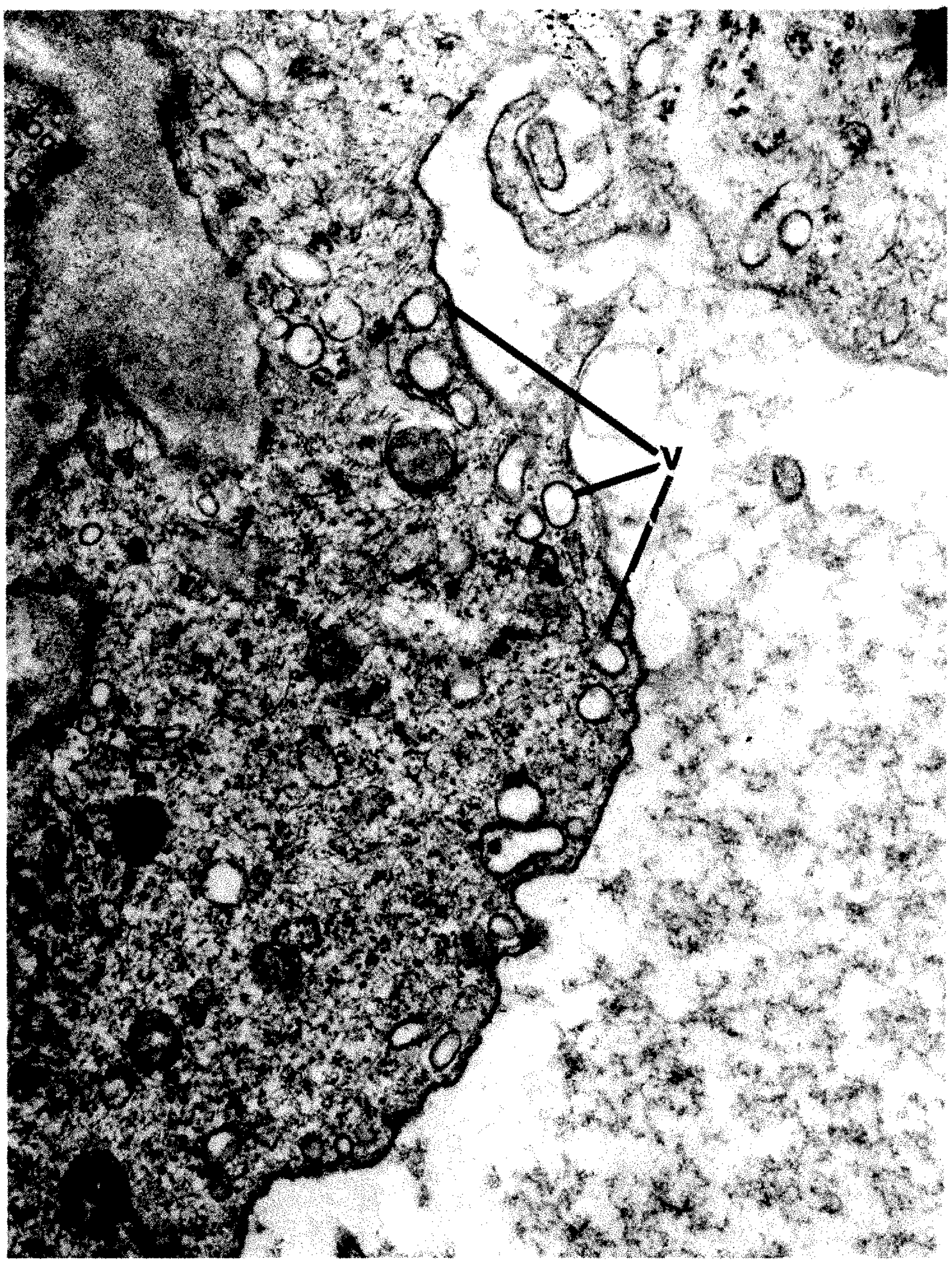

Fig. 4. Part of an endothelial cell of endometrium obtained after treatment with 1000 mg of naproxen daily on the day of onset of menstruation. The cytoplasmic matrix is less dense than shown in Fig. 3 representing the control endometrium. The lysosome *(L)* shows a higher density than in the control endometrium and the plasmolemmal vesicles *(v)* are found in a significantly increased number in the periphery of the cytoplasm. (Original magnification ×55,400.)

phosphate–induced platelet aggregation. Since it has also been shown that an inhibition of this adenosine 5′-diphosphate–induced platelet aggregation is associated with a rise of cyclic adenosine monophosphate,[21] it cannot be excluded that the increased number of plasmolemmal vesicles per square micrometer observed in the present study after the naproxen treatment is reflecting a mechanism similar to that observed in the mice treated with cyclic adenosine monophosphate.

In spite of the facts that less regressive changes were observed in the endometrium obtained in the treatment cycles than in the control cycles and that a more active intracellular transport of water-soluble substance seemed to exist in the treated cycles, the administration of 1000 mg of naproxen daily during the late secretory phase did not seem to prevent the onset of the menstrual bleeding. The mechanism of menstruation therefore still remains an open question and further studies are required to clarify the factors responsible for the onset of menstruation.

We are grateful for the assistance of Ingalill Backlund and Astrid Häggblad.

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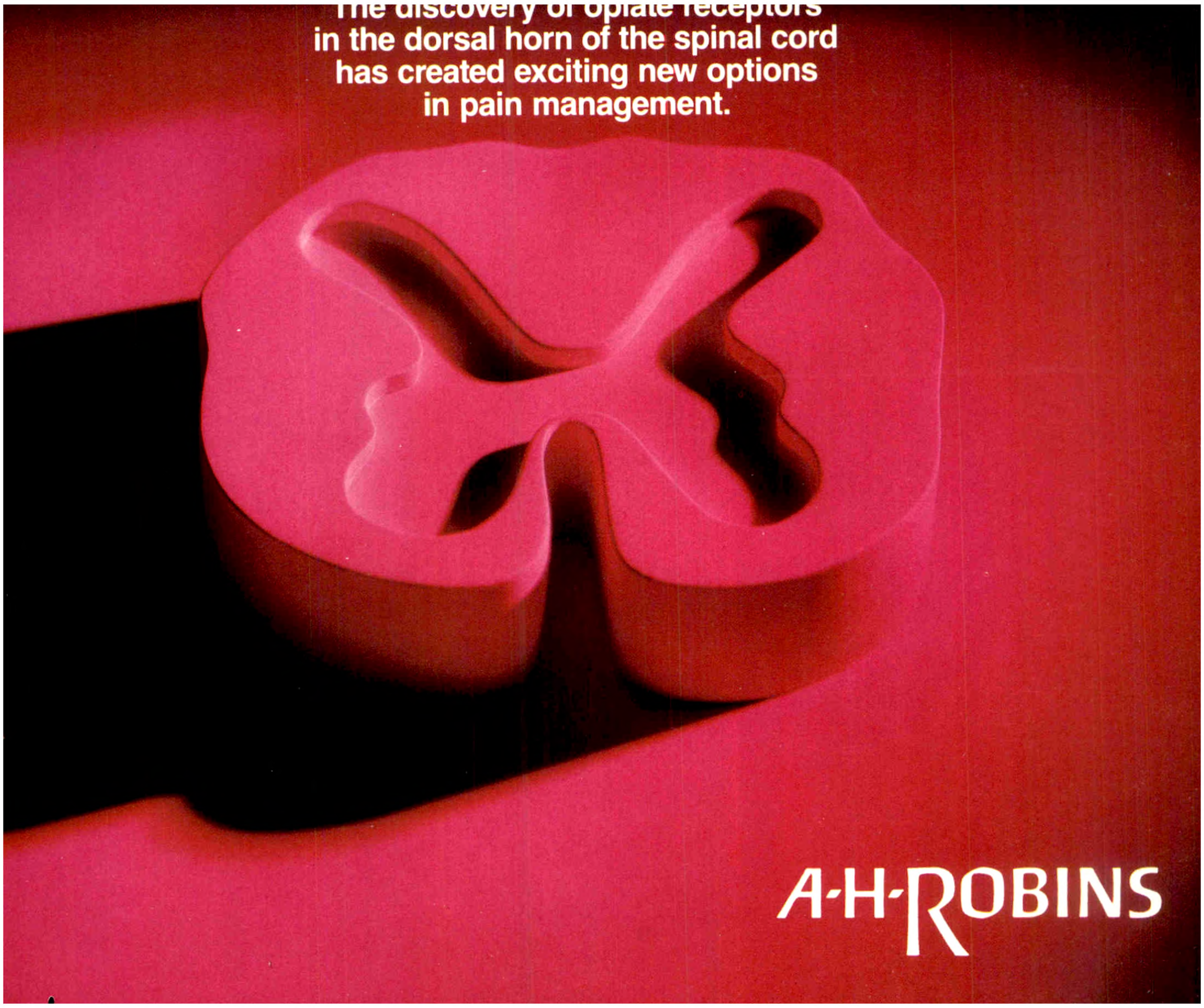
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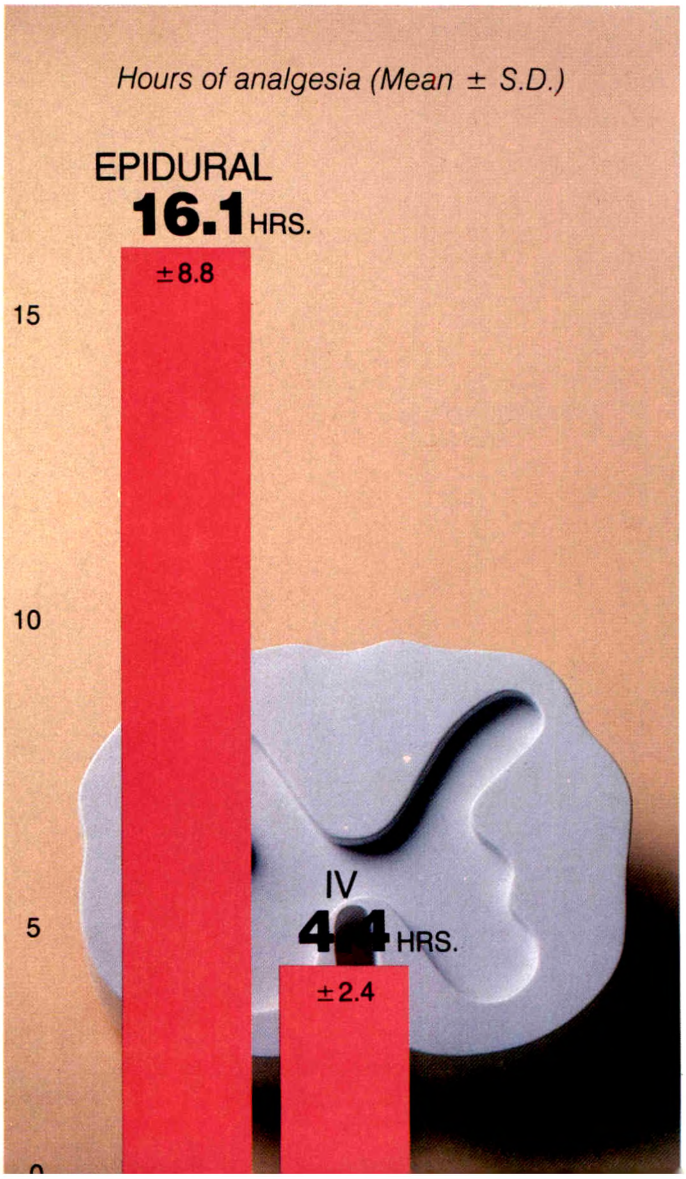

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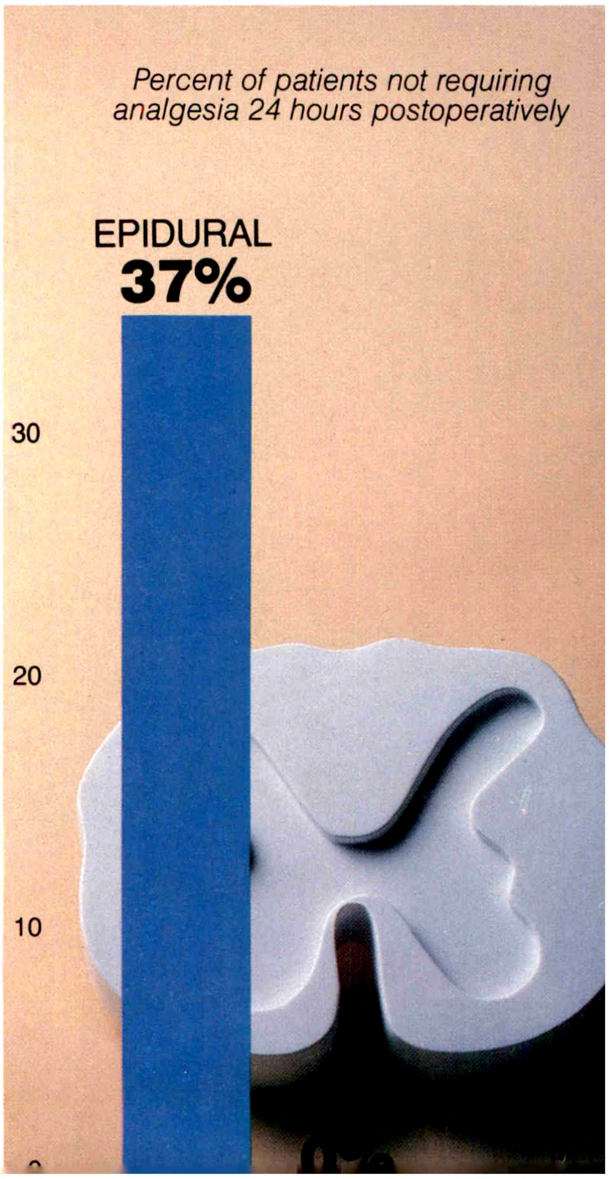

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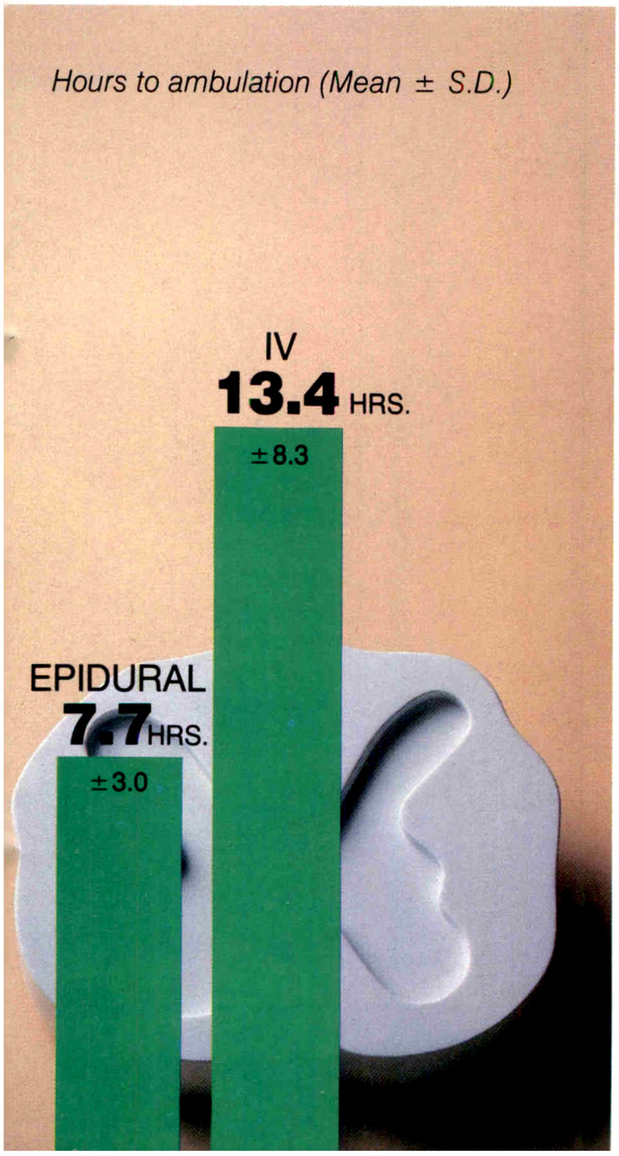
*Charts depict the results of a double-blind study comparing the analgesic efficacy of 5 mg epidural morphine (N = 19) with that of 5 mg intravenous (IV) morphine (N = 19) following cesarean section.**



- **Virtually no sedation or loss of motor function**
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*Cohen SE, Woods WA: Anesthesiology 58:500, 1983

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INDICATIONS AND USAGE

Preservative-free DURAMORPH® PF is a systemic narcotic analgesic for administration by the intravenous, epidural or intrathecal routes. It is used for the management of pain not responsive to non-narcotic analgesics. Morphine sulfate, administered epidurally or intrathecally, provides pain relief for extended periods without attendant loss of motor, sensory or sympathetic function.

CONTRAINDICATIONS

DURAMORPH® PF is contraindicated in those medical conditions which would preclude the administration of opioids by the intravenous route—allergy to morphine or other opiates, acute bronchial asthma, upper airway obstruction.

Administration of morphine by the epidural or intrathecal route is contraindicated in the presence of infection at the injection site, anticoagulant therapy, bleeding diathesis, parenterally administered corticosteroids within a two week period or other concomitant drug therapy or medical condition which would contraindicate the technique of epidural or intrathecal analgesia.

WARNINGS

DURAMORPH® PF administration should be limited to use by those familiar with the management of respiratory depression, and in the case of epidural or intrathecal administration, familiar with the techniques and patient management problems associated with epidural or intrathecal drug administration. Because epidural administration has been associated with lessened potential for immediate or late adverse effects than intrathecal administration, the epidural route should be used whenever possible. Rapid intravenous administration may result in chest wall rigidity.

FACILITIES WHERE DURAMORPH® PF IS ADMINISTERED MUST BE EQUIPPED WITH RESUSCITATIVE EQUIPMENT, OXYGEN, NALOXONE INJECTION, AND OTHER RESUSCITATIVE DRUGS. WHEN THE EPIDURAL OR INTRATHECAL ROUTE OF ADMINISTRATION IS EMPLOYED, PATIENTS MUST BE OBSERVED IN A FULLY EQUIPPED AND STAFFED ENVIRONMENT FOR AT LEAST 24 HOURS.

SEVERE RESPIRATORY DEPRESSION UP TO 24 HOURS FOLLOWING EPIDURAL OR INTRATHECAL ADMINISTRATION HAS BEEN REPORTED.

Morphine sulfate may be habit forming. (See Drug Abuse and Dependence section.)

PRECAUTIONS

GENERAL

Preservative-free DURAMORPH® PF (Morphine Sulfate Injection, USP) should be administered with extreme caution in aged or debilitated patients, in the presence of increased intracranial/intraocular pressure and in patients with head injury. Pupillary changes (miosis) may obscure the course of intracranial pathology. Care is urged in patients who have a decreased respiratory reserve (e.g., emphysema, severe obesity, kyphoscoliosis).

Seizures may result from high doses. Patients with known seizure disorders should be carefully observed for evidence of morphine-induced seizure activity.

It is recommended that administration of DURAMORPH® PF by the epidural or intrathecal routes be limited to the lumbar area. Intrathecal use has been associated with a higher incidence of respiratory depression than epidural use.

Smooth muscle hypertonicity may result in biliary colic, difficulty in urination and possible urinary retention requiring catheterization. Consideration should be given to risks inherent in urethral catheterization, e.g., sepsis, when epidural or intrathecal administration is considered, especially in the perioperative period.

Elimination half-life may be prolonged in patients with reduced metabolic rates and with hepatic or renal dysfunction. Hence, care should be exercised in administering morphine in these conditions, particularly with repeated dosing.

Patients with reduced circulating blood volume, impaired myocardial function or on sympatholytic drugs should be observed carefully for orthostatic hypotension, particularly in transport.

Patients with chronic obstructive pulmonary disease and patients with acute asthmatic attack may develop acute respiratory failure with administration of morphine. Use in these patients should be reserved for those whose conditions require endotracheal intubation and respiratory support or control of ventilation.

DRUG INTERACTIONS

Depressant effects of morphine are potentiated by either concomitant administration or in the presence of other CNS depressants such as alcohol, sedatives, antihistaminics or psychotropic drugs (e.g., MAO inhibitors, phenothiazines, butyrophenones and tricyclic antidepressants). Premedication or intra-anesthetic use of neuroleptics with morphine may increase the risk of respiratory depression.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Studies of morphine sulfate in animals to evaluate the carcinogenic and mutagenic potential or the effect on fertility have not been conducted.

PREGNANCY

Teratogenic effects—Pregnancy Category C. Animal reproduction studies have not been conducted with morphine sulfate. It is also not known whether morphine sulfate can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Morphine sulfate should be given to a pregnant woman only if clearly needed.

Nonteratogenic effects. Infants born from mothers who have been taking morphine chronically may exhibit withdrawal symptoms.

LABOR AND DELIVERY

Intravenous morphine readily passes into the fetal circulation and may result in respiratory depression in the neonate. Naloxone and resuscitative equipment should be available for reversal of narcotic-induced respiratory depression in the neonate. In addition, intravenous morphine may reduce the strength, duration and frequency of uterine contraction resulting in prolonged labor.

Epidurally and intrathecally administered morphine readily passes into the fetal circulation and may result in respiratory depression of the neonate. Controlled clinical studies have shown that *epidural* administration has little or no effect on the relief of labor pain.

However, studies have suggested that in most cases 0.2 to 1 mg of morphine *intrathecally* provides adequate pain relief with little effect on the duration of first stage labor. The second stage labor, though, may be prolonged if the parturient is not encouraged to bear down. A continuous intravenous infusion of naloxone, 0.6 mg/hr, for 24 hours after intrathecal injection may be employed to reduce the incidence of potential side effects.

NURSING MOTHERS

Morphine is excreted in maternal milk. Effect on the nursing infant is not known.

PEDIATRIC USE

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

The most serious side effect is respiratory depression. Because of delay in maximum CNS effect with intravenously administered dru (30 min), rapid administration may result in overdosing. Bol administration by the epidural or intrathecal route may result early respiratory depression due to direct venous redistribution morphine to the respiratory centers in the brain. Late (up to 2 hours) onset of acute respiratory depression has been reporte with administration by the epidural or intrathecal route and believed to be the result of rostral spread. Reports of respirato depression following intrathecal administration have been mo frequent, but the dosage used in most of these cases has bee considerably higher than that recommended. This depression ma be severe and could require intervention (See Warnings and Ove dosage sections). Even without clinical evidence of ventilato inadequacy, a diminished CO_2 ventilation response may be note for up to 22 hours following epidural or intrathecal administratio

While low doses of intravenously administered morphine have litt effect on cardiovascular stability, high doses are excitatory, resul ing from sympathetic hyperactivity and increase in circulati catecholamines. Excitation of the central nervous system resulti in convulsions may accompany high doses of morphine giv intravenously. Dysphoric reactions may occur and toxic psychos have been reported.

Epidural or intrathecal administration is accompanied by a hi incidence of pruritus which is dose related but not confined to s of administration. Nausea and vomiting are frequently seen patients following morphine administration. Urinary retentic which may persist for 10-20 hours following single epidural intrathecal administration has been reported in approximately 90 of males. Incidence is somewhat lower in females. Patients m require catheterization (see Precautions). Pruritus, nause vomiting and urinary retention frequently can be alleviated by t intravenous administration of low doses of naloxone (0.2 mg).

Tolerance and dependence to chronically administered morphin by whatever route, is known to occur (see Drug Abuse and Depe dence section).

Miscellaneous side effects include constipation, headache, anxie depression of cough reflex, interference with thermal regulati and oliguria. Evidence of histamine release such as uticaria, whe and/or local tissue irritation may occur.

In general, side effects are amenable to reversal by narcotic anta onists. NALOXONE INJECTION AND RESUSCITATIVE EQUIPME SHOULD BE IMMEDIATELY AVAILABLE FOR ADMINISTRATION CASE OF LIFE-THREATENING OR INTOLERABLE SIDE EFFECT

DRUG ABUSE AND DEPENDENCE

Controlled Substance: Morphine sulfate is a Schedule II substan under the Drug Enforcement Administration classification.

Abuse: Morphine has recognized abuse potential.

Dependence: Cerebral and spinal receptors may develop tol ance/dependence independently, as a function of local dosag Care must be taken to avert withdrawal in those patients who ha been maintained on parenteral/oral narcotics when epidural intrathecal administration is considered. Withdrawal may occ following chronic epidural or intrathecal administration, as well the development of tolerance to morphine by these routes. (S Nonteratogenic effects under Pregnancy.)

OVERDOSAGE

Overdosage is characterized by respiratory depression with without concomitant CNS depression. Since respiratory arrest m result either through direct depression of the respiratory center as the result of hypoxia, primary attention should be given to t establishment of adequate respiratory exchange through provisi of a patent airway and institution of assisted or controlled venti tion. The narcotic antagonist, naloxone, is a specific antido Naloxone (usually 0.4 mg) should be administered intravenous simultaneously with respiratory resuscitation. *As the duration effect of naloxone is considerably shorter than that of epidural intrathecal morphine, repeated administration may be necessa* Patients should be closely observed for evidence of renarcotizatic *Note: Respiratory depression may be delayed in onset up to hours* following epidural or intrathecal administration. In pain conditions, reversal of narcotic effect may result in acute onset pain and release of catecholamines. Careful administration naloxone may permit reversal of side effects without affecti analgesia. Parenteral administration of narcotics in patients rece ing epidural or intrathecal morphine may result in overdosage.

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Human endometrial prostaglandin E_2 binding sites and their profiles during the menstrual cycle and in pathologic states

G. E. Hofmann, M.D., Ph.D., Ch. V. Rao, Ph.D., F. D. De Leon, M.D., A. A. Toledo, M.D., and J. S. Sanfilippo, M.D.
Louisville, Kentucky

Endometrial tissue from uteri of 35 nonpregnant, premenopausal women was assayed for prostaglandin E_2 and $F_{2\alpha}$ binding site content as a function of the phase of the menstrual cycle and the pathologic state. For all specimens, tritium-labeled prostaglandin $F_{2\alpha}$ binding was very low (<8 fmol/mg of protein) or undetectable regardless of the phase of the menstrual cycle or pathologic state or in the presence or absence of 10 μmol/L of indomethacin, a prostaglandin synthetase inhibitor. However, tritium-labeled prostaglandin E_2 binding was detected in every specimen and was independent of the presence or absence of indomethacin. Binding of tritium-labeled prostaglandin E_2, as determined by Scatchard analyses, was biphasic (dissociation constant ~ 1 nmol/L; dissociation constant for low-affinity sites ~ 10 nmol/L) for both proliferative and secretory endometrial tissue. However, the total number of prostaglandin E_2 binding sites, determined from Scatchard or single-point analyses, was significantly higher ($p < 0.01$) in proliferative endometrium compared to secretory endometrium. In addition, for endometrium from the proliferative phase of the menstrual cycle, the diagnosis of abnormal uterine bleeding was associated with higher ($p < 0.01$) tritium-labeled prostaglandin E_2 binding than diagnosis of dysmenorrhea, stress urinary incontinence and uterine prolapse, or pelvic inflammatory disease. Endometrial specimens with the last four diagnoses did not differ significantly ($p > 0.1$) from each other. (AM J OBSTET GYNECOL 1985; 151:369-75.)

Key words: Prostaglandin receptors, endometrium, menstrual cycle, gynecologic conditions

Since the discovery and characterization of prostaglandins,[1] their role in uterine pathophysiologic conditions has received considerable attention. Prostaglandin E (PGE) and $F_{2\alpha}$ ($PGF_{2\alpha}$) are potent stimulators of human uterine smooth muscle contractions,[2,3] and this action is presumably mediated through binding to discrete PGE and $PGF_{2\alpha}$ binding sites in myometrium.[4,5] The myometrial prostaglandin binding sites have been well characterized.[4-7] Myometrial prostaglandin binding sites exhibit topography, i.e., higher levels in the fundus which decreases toward the cervical end in parallel with smooth muscle content.[4] This finding suggested that human uterine prostaglandin binding sites are primarily confined to myometrium.[4] The question of obvious importance is whether human endometrium possesses any prostaglandin binding sites. There are no data in the literature to answer this question. In fact, one previous study reported a lack of prostaglandin binding sites in the endometrium of the rhesus monkey.[6] The present study was undertaken to answer the following questions: (1) Does human endometrium contain prostaglandin binding sites? (2) Do the binding site levels vary with the phase of the menstrual cycle? (3) Do uterine pathologic conditions alter the binding site levels?

Material and methods

Material. The tritium-labeled prostaglandins were purchased from New England Nuclear, Boston, Massachusetts, or Amersham Corporation, Arlington Heights, Illinois. Specific activities were: tritium-labeled PGE_2, 160 Ci/mmol, and tritium-labeled $PGF_{2\alpha}$, 150 Ci/mmol. The purity of the tritium-labeled prostaglandins was checked by thin-layer chromatography, and if it was less than 95%, they were purified as described previously.[8] Aliquots of tritium-labeled prostaglandin stocks were diluted to about 1 μCi/ml with absolute redistilled ethanol and stored at −20° C between uses.

Unlabeled prostaglandins were purchased from The Upjohn Company, Kalamazoo, Michigan; bovine serum albumin (type V) and indomethacin, from Sigma Chemical Company, St. Louis, Missouri; and the 0.45 μm pore size filters, from International Research Distributors, Dayton, Ohio. All other chemicals were purchased from commercial sources.

Methods

Tissue collection and preparation. Uteri removed for medically indicated reasons from nonpregnant pre-

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Reprint requests: Dr. Ch. V. Rao, Department of Obstetrics and Gynecology, 437 MDR Building, University of Louisville, Health Sciences Center, Louisville, KY 40292.

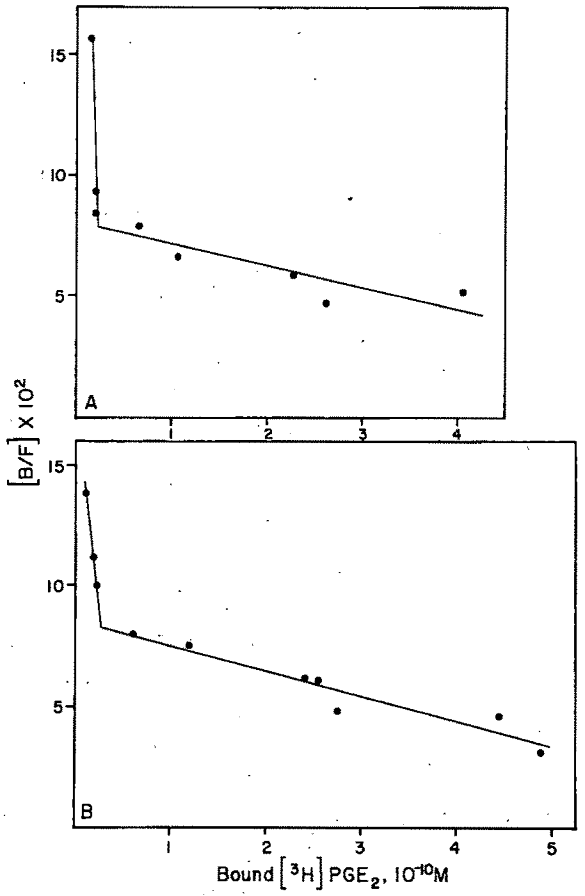

Fig. 1. Scatchard analyses of tritium-labeled PGE_2 binding to proliferative *(A)* and secretory *(B)* endometrium. The dissociation constants and number of sites calculated (see Methods section) are presented in Table III.

menopausal women (age range, 20 to 48 years) were obtained from the operating room and immediately placed in ice-cold physiologic saline. After examination by the pathologist, they were frozen at −90° C. Endometrium was obtained by gentle scraping of the uterine cavity with a small spatula.

Immediately prior to binding experiments, endometrium was homogenized at 4° C in 10 mmol/L of Tris hydrochloride, pH 7.0, containing 250 mmol/L of sucrose, 1 mmol/L of calcium chloride, 1 mmol/L dithiothreitol, and 0.1% gelatin, with two 15-second bursts on a Brinkmann polytron homogenizer (Brinkmann Instruments, Westbury, New York). The homogenates were centrifuged at 10,000 × g for ten minutes, and the pellet, which was mostly free of hemoglobin, was resuspended in homogenization buffer. Protein concentration was determined after digestion at 80° C in 0.1 mol/L of sodium hydroxide containing 0.1% sodium dodecyl sulphate by the method of Lowry et al.[9] with bovine serum albumin used as the standard.

Binding studies. Tritium-labeled prostaglandin binding was assayed according to the conditions that have been found to be optimal for bovine luteal prostaglandin binding sites.[10] These conditions, in general, are similar to prostaglandin binding sites in other tissues.

Briefly, tritium-labeled prostaglandins in redistilled ethanol were pipetted into 12 by 75 mm disposable glass tubes. The ethanol was blown dry under a stream of nitrogen. Aliquots of fractions (200 to 500 μg of protein) were then added in amounts of 0.1 ml to the tubes and incubated at 38° C for 1 hour. The incubation buffer composition was the same as that of the homogenizing buffer and the final tritium-labeled prostaglandin concentration during the incubation was 5 nmol/L. Following incubation, 1.0 ml of 10 mmol/L Tris hydrochloride buffer, pH 7.0, at 4° C was added to each tube and poured on Metricel filters of 0.45 μm pore size positioned on a Millipore manifold under a vacuum. The tubes were rinsed twice with 1 ml aliquots of 10 mmol/L Tris hydrochloride buffer and filtered. Finally, each filter was washed with 10 ml of Tris hydrochloride buffer at 4° C. The filters were then cut into halves and placed into scintillation vials containing 10 ml of scintillation fluid and counted in a Packard Tricarb liquid scintillation counter having a counting efficiency of 50% for tritium. The scintillation fluid consisted of toluene/Triton X-100/Packard Permafluor (25X), 55:18:6 (v/v). Nonspecific binding was determined for each specimen in parallel in the presence of 30 μmol/L of corresponding unlabeled prostaglandins and was subtracted from the total binding to obtain specific binding.

Although the ability of other unlabeled prostaglandins and prostaglandin metabolites to inhibit tritium-labeled PGE_2 binding to endometrium was not examined, it is more than likely that unlabeled PGE_1 would have, while other prostaglandins and prostaglandin metabolites would not have, competed for tritium-labeled PGE_2 binding, from experience with PGE receptors in human myometrium[4, 7] and in a variety of other tissues.[11]

To evaluate whether the observed tritium-labeled prostaglandin binding was being influenced by prostaglandin synthesis during the binding reaction, tritium-labeled prostaglandin binding to some of the specimens was determined in the presence and absence of 10 μmol/L of indomethacin, a potent prostaglandin synthetase inhibitor.

Two endometrial specimens, one each for proliferative and secretory phases, were incubated at 38° C for 1 hour with 0.1 to 30 nmol/L of tritium-labeled PGE_2 in the presence and absence of 30 μmol/L of unlabeled PGE_2. The specific binding data were transformed according to Scatchard[12] and graphically displayed in Fig. 1. As can be seen in this figure, the Scatchard plots are curvilinear. This can be due to the presence of two

Table I. Tritium-labeled prostaglandin binding to human endometrium as a function of the phase of the menstrual cycle and the pathologic state

Patient No.	*Phase of menstrual cycle*	*Diagnosis*	*Tritium-labeled PGE_2 bound (fmol/mg protein, mean ± SE)*	*Tritium-labeled $PGF_{2\alpha}$ bound (fmol/mg protein, mean ± SE)*
1	P	AUB	84.9 ± 11.8	ND
2	P	DYS	25.3 ± 0.8	ND
3	P	AUB/ENDO	35.2 ± 1.2	ND
4	P	ENDO	100.0 ± 10.3*	ND
5	P	SUI	26.7 ± 7.5†	ND
6	P	AUB	73.4 ± 8.3	ND
7	P	DYS	116.9 ± 9.9	ND
8	P	AUB	93.1 ± 13.4	ND
9	P	PID	30.8 ± 5.6	4.2 ± 1.8
10	P	AUB	41.8 ± 15.9	ND
11	P	PID	44.9 ± 7.4	ND
12	P	UP	33.4 ± 6.6	3.4 ± 3.0
13	P	AUB	50.5 ± 4.3	ND
14	P	DYS/ENDO	28.2 ± 1.0	ND
15	P	DYS	25.6 ± 14.6	ND
16	P	DYS	41.3 ± 4.2	ND
17	P	AUB	48.1 ± 1.8	ND
18	P	DYS	32.7 ± 3.7	8.3 ± 2.2
19	P	UP	27.2 ± 5.2	ND
20	P	UP	48.6 ± 5.3	ND
21	P	AUB	72.3 ± 8.8	ND
22	P	PID	64.3 ± 8.5	ND
23	P	UP	18.3 ± 6.2	ND
24	P	PID	52.2 ± 10.5	ND
25	S	UP	13.9 ± 1.4	ND
26	S	DYS	14.9 ± 1.5	ND
27	S	AUB	25.6 ± 14.5	4.1 ± 1.5
28	S	AUB	7.6 ± 3.1	5.0 ± 2.8
29	S	UP	27.7 ± 4.7	ND
30	S	AUB	23.3 ± 1.3	3.1 ± 2.1
31	S	SUI	24.9 ± 3.7	ND
32	S	OC	22.1 ± 7.5	ND
33	S	AUB	17.4 ± 6.3	ND
34	S	PCOD	7.7 ± 3.3	ND
35	S	ENDO	20.7 ± 5.9	ND

The abbreviations used are: ND, none detected; P, proliferative; S, secretory; AUB, abnormal uterine bleeding; DYS, dysmenorrhea; ENDO, endometriosis; SUI, stress urinary incontinence; PID, pelvic inflammatory disease; UP, uterine prolapse; PCOD, polycystic ovarian disease; OC, ovarian cyst.

*Patient taking Lo/Ovral at time of hysterectomy.

†Patient taking Norinyl 1:50 at time of hysterectomy.

independent sets of binding sites or to negative cooperativity. The latter could not be tested because of the limited availability of endometrial scrapings.

The prostaglandin receptors in a wide variety of tissues exhibit a curvilinear Scatchard plot.[11] There is only one study in the literature which examined this and found that heterogeneous tritium-labeled prostaglandin binding was not due to negative cooperativity.[13] This led us to assume that heterogeneous tritium-labeled PGE_2 binding in the present studies was also not due to negative cooperativity. If this assumption is incorrect, then the dissociation constant of high-affinity sites approximates that of a single set of sites in negative cooperativity and the sum of high- and low-affinity sites equals the total number of sites in negative cooperativity.

Least-square fit analysis,[14] which corrects for low-affinity line contribution to high-affinity line intercepts, was used to determine the slope and intercepts. The apparent dissociation constants were calculated from the reciprocal of the slopes and the total number of available sites from the X-axis intercepts. The X-axis intercepts of corrected high- and low-affinity lines gave values for high-affinity and total number of available PGE_2 binding sites, respectively. The difference between these two lines gave a value for the number of available low-affinity binding sites. The lines in Fig. 1 were drawn according to the actual data points for the sake of simplicity.

Specimen dating and diagnosis. The phase of the menstrual cycle was determined by the patient's last normal menstrual period and histopathologic evalua-

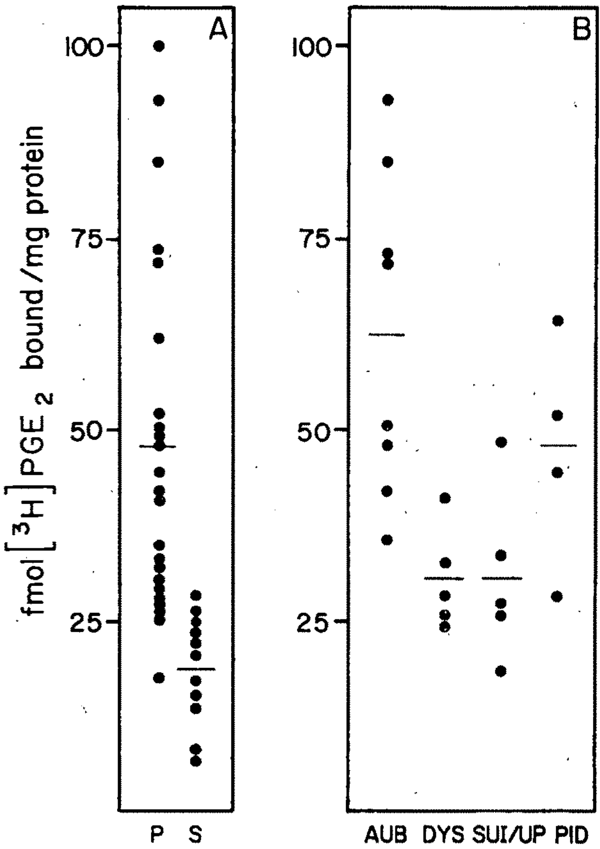

Fig. 2. *A*, Graphic display of tritium-labeled PGE_2 binding to 34 endometrial specimens by phase of the menstrual cycle. *B*, tritium-labeled PGE_2 binding to 22 proliferative endometrial specimens by diagnosis. *Horizontal bars* represent the mean of each group. *P*: Proliferative; *S*: secretory phase of the menstrual cycle; *AUB*: abnormal uterine bleeding; *DYS*: dysmenorrhea; *SUI/UP*: stress urinary incontinence and uterine prolapse; *PID*: pelvic inflammatory disease.

Table II. Tritium-labeled PGE_2 binding to endometrium in the absence and presence of indomethacin

Patient No.	*Tritium-labeled PGE_2 binding (fmol/mg protein)*	
	No indomethacin	*10 μmol/L indomethacin*
1	17.4 ± 6.3	18.6 ± 5.8
2	7.7 ± 3.3	4.9 ± 2.6
3	18.3 ± 6.2	20.5 ± 4.5
4	15.7 ± 2.4	20.4 ± 4.3
5	52.2 ± 10.5	47.7 ± 5.0
6	20.7 ± 5.9	20.8 ± 6.4

Tritium-labeled $PGF_{2\alpha}$ binding was not detected in any of the six endometrial specimens in either the absence or presence of indomethacin.

Table III. The dissociation constants and number of sites calculated from Scatchard plots of tritium-labeled PGE_2 binding to proliferative and secretory endometrium

Parameter	*Proliferative*	*Secretory*
Correlation coefficient*	0.99	0.99
Kd_1† (nmol/L)	1.0	0.3
N_1‡ (fmol/mg protein)	33.8	5.6
Correlation coefficient	0.88	0.95
Kd_2 (nmol/L)	11.0	9.5
N_2 (fmol/mg protein)	372.2	160.0

*Goodness of fit of data points to the straight line. Maximum value = 1.0

†Kd = Apparent dissociation constant. Subscripts 1 and 2 refer to high- and low-affinity sites, respectively.

‡N = Total number of available sites.

tion. The diagnosis was taken from the patient's chart.

Statistical analysis. The data in Tables I and II are the means and their standard errors of at least quadruplicate determinations. Least-squares curve fit analysis, Wilcoxon rank sum test with 95% confidence limits, and correlation coefficients were used in the analysis of some of the binding data.[15]

Results

Thirty-five endometrial specimens were examined for specific binding of tritium-labeled PGE_2 and $PGF_{2\alpha}$. Tritium-labeled $PGF_{2\alpha}$ binding in all specimens was either very low (<8 fmol/mg of protein) or undetectable (Table I). Tritium-labeled PGE_2 on the other hand, was detected in all specimens. Table II demonstrates that the presence or absence of 10 μmol/L of indomethacin, a potent prostaglandin synthetase inhibitor, had no effect on the level of tritium-labeled PGE_2 binding or the detectability of tritium-labeled $PGF_{2\alpha}$ binding.

Fig. 1 shows that the Scatchard plots of tritium-labeled PGE_2 specific binding to proliferative *(A)* and secretory *(B)* endometrium were curvilinear. Table III shows that the apparent dissociation constants calculated for high- and low-affinity sites in proliferative and secretory endometrium were similar. However, the proliferative endometrium contained a greater number of sites than the secretory endometrium.

The dissociation constant of high-affinity endometrial sites is very similar to that of human myometrium[4] and leiomyoma.[5] The dissociation constant of low-affinity endometrial sites, on the other hand, is lower (affinity is higher) than the corresponding ones in myometrium and leiomyoma.[4, 5]

Fig. 2, *A*, shows that proliferative endometrium (n = 24) bound 2.7 times more tritium-labeled PGE_2 than did secretory endometrium (n = 11) (proliferative: mean = 50.6 fmol/mg of protein; range, 18.3 to 116.9; secretory: mean = 18.7 fmol/mg of protein; range, 7.5 to 27.7; $p < 0.01$).

Fig. 2, *B*, shows that proliferative* endometrium

*Insufficient numbers of endometrial specimens from the secretory phase of the menstual cycle did not permit us to compare tritium-labeled PGE_2 binding by diagnosis.

from patients with the diagnosis of abnormal uterine bleeding (mean = 62.4 fmol/mg of protein; range, 35.2 to 93.1; n = 8) bound significantly ($p < 0.01$) more tritium-labeled PGE_2 than did endometrium from patients with a diagnosis of dysmenorrhea* (mean = 30.6 fmol/mg of protein; range, 25.3 to 41.8; n = 5), stress urinary incontinence/uterine prolapse (mean = 30.8 fmol/mg of protein; range, 18.3 to 48.6; n = 5) and pelvic inflammatory disease (mean = 48.0 fmol/mg of protein; range, 30.8 to 64.3; n = 4). Differences between the last three groups did not reach statistical significance ($p > 0.1$). The stress urinary incontinence/uterine prolapse group may be considered normal as they have no specific uterine abnormality.

Comment

This study demonstrates for the first time that human endometrium contains detectable PGE and very few or no detectable $PGF_{2\alpha}$ binding sites. Three possibilities were considered for very little or no $PGF_{2\alpha}$ binding observed in human endometrium: (1) There was masking of binding sites by endogenously produced $PGF_{2\alpha}$. If this were the case, then there should have been detectable binding during the proliferative phase when endometrial $PGF_{2\alpha}$ production was low[16] and nondetectable binding during the secretory phase when endometrial $PGF_{2\alpha}$ production was high.[16] Contrary to this expectation, $PGF_{2\alpha}$ binding was very slight to nondetectable regardless of the phase of the cycle. Another line of evidence that might argue against the above possibility is that endometrial PGE_2 and $PGF_{2\alpha}$ production is about the same,[17] which should make both prostaglandin binding sites nondetectable unless $PGF_{2\alpha}$ binding sites are very few to begin with. (2) Rapid endometrial metabolism of tritium-labeled $PGF_{2\alpha}$ lowered the concentration of bindable ligand. This is not a viable explanation because endometrial metabolism of $PGF_{2\alpha}$ to 13,14-dihydro-$PGF_{2\alpha}$ or 13,14-dihydro-15-keto-$PGF_{2\alpha}$ is negligible.[18] (3) There was masking of binding sites of $PGF_{2\alpha}$ produced during homogenization and centrifugation procedures. If this possiblity were to be true, then there should be greater production of $PGF_{2\alpha}$ than of PGE_2 during the above steps at 4° C to explain the lack of and the presence of endometrial $PGF_{2\alpha}$ and PGE_2 binding sites, respectively. To our knowledge, there are no data for such a possibility in any tissue. (4) There was masking of binding sites by $PGF_{2\alpha}$ produced during the binding reaction. The similar binding in the presence and absence of indomethacin (Table II) rules out this possibility. These points reinforce the belief that binding sites are not masked but rather that human endometrium indeed possesses very few or no detectable $PGF_{2\alpha}$ binding sites. The $PGF_{2\alpha}$ produced by endometrium may then serve to act on myometrium or other adjacent tissues which can be reached without going through the general circulation.

Possible myometrial contamination of the endometrium should be considered before one can ascertain whether endometrial prostaglandin E binding sites are intrinsic to the endometrium. First of all, endometrium was gently scraped from the uterus to avoid myometrial contamination. Even if some myometrium was inadvertently scraped with endometrium, endometrial prostaglandin binding sites would still be intrinsic for the following reasons: (1) While there was no detectable cyclic variation in myometrial prostaglandin E binding sites,[4] there was a definite cyclic variation in endometrial prostaglandin E binding sites; (2) the prostaglandin E binding site levels in endometrium in many cases were higher than the highest levels reported for myometrium[4, 5]; (3) the tritium-labeled PGE_2 binding was not the same in myometrium[4] and endometrium in various gynecologic conditions (myometrium: binding in the group with abnormal uterine bleeding was the same as that of the stress urinary incontinence group but higher than that of the dysmenorrhea group; endometrium binding in the group with abnormal uterine bleeding was higher than that of both the group with stress urinary incontinence and the group with dysmenorrhea); (4) myometrium always contained $PGF_{2\alpha}$ binding sites,[4] whereas endometrium contained these binding sites only occasionally.

Thirty-five endometrial specimens were assayed for tritium-labled prostaglandin binding. Neither the amounts of endometrial scrapings available nor the cost of tritium-labeled prostaglandins allowed us to determine by saturation analysis the total number of prostaglandin binding sites present in every endometrial specimen. Therefore, all specimens were examined for relative binding differences with the use of a single subsaturating concentration of tritium-labled prostaglandin, that is, 5 nmol/L, which is in the middle of dissociation constants of high- and low-affinity sites. After this binding evaluation and upon observing tritium-labeled PGE_2 binding differences between proliferative and secretory phase endometrium, we then examined whether the above difference was due to changes in binding site affinities and/or in the total number of available binding sites. The results revealed that while the affinities of PGE_2 binding sites were similar, the total number of available sites was higher in proliferative endometrium than in secretory endometrium. The above approach has been previously used to get around the problems such as described at the beginning of this paragraph.[19] This approach has worked extremely well in the present studies, because the mean proliferative endometrial binding as determined by use of 5 nmol/L of tritium-labeled PGE_2 was 2.7 times higher than that of secretory endometrium. This number is in excellent agreement with the value of 2.45 times calculated from the total (high- and low-affinity) number of available sites from the Scatchard plots.

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*The value of 116.9 fmol/mg of protein for one patient with the diagnosis of dysmenorrhea was omitted from statistical analysis and from Fig. 2 because it was 2.8 times greater than the next largest value found for this group. The binding measurement could not be repeated because of the small size of the specimen. Hence, it is unknown if this was an experimental error or represented an unusual level of PGE_2 binding sites in the endometrium of this particular patient.

The higher tritium-labeled PGE_2 binding in proliferative phase endometrium and the lower binding in secretory phase endometrium parallels the endometrial prostaglandin E production profiles.[16, 17] These data, along with the observation that the presence or absence of indomethacin had no effect on the level of tritium-labeled PGE_2 binding, suggests that cyclic variation or even individual sample variation could not have been entirely due to differential binding site masking by prostaglandin E produced endogenously. Since the uterine specimens used in these studies were not quite "normal," tritium-labeled PGE_2 binding differences seen between proliferative and secretory phase endometrium may or may not be strictly relevant to normal uterine physiologic characteristics.

It is not known what cell types in human endometrium are responsible for prostaglandin binding. It is possible, however, that a greater number of binding sites for a particular cell population or a greater number of particular cells each with the same number of binding sites could explain the higher tritium-labeled PGE_2 binding in proliferative endometrium than in secretory endometrium.

The proliferative endometrium from patients with a diagnosis of abnormal uterine bleeding bound more tritium-labeled PGE_2 than endometrium from patients with a diagnosis of dysmenorrhea, stress urinary incontinence/uterine prolapse, or pelvic inflammatory disease. The binding in the last three groups was not significantly different. The stress urinary incontinence/uterine prolapse group may be considered normal, as they had no specific uterine abnormality. As previously suggested, the increased levels of prostaglandin E and its binding sites in myometrium (and now in endometrium as well) of patients with abnormal uterine bleeding may lead to increased vascular smooth muscle relaxation and excessive bleeding.[4]

The physiologic significance of prostaglandin E binding sites in endometrial tissue is not known. Obviously, they will not be relevant to uterine contractions. Prostaglandins, particularly of the E series, have been shown to increase endometrial vascular permeability and subsequent decidualization in various animal species.[20-23] If prostaglandin E has a similar role in humans, then prostaglandin E and its binding sites can be expected to influence the above and other endometrial events which are prerequisite for the successful implantation of a blastocyst.

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Is prostaglandin E_2 really of therapeutic value for postoperative urinary retention? Results of a prospectively randomized double-blind study

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In a prospectively randomized double-blind study 28 patients with urinary retention after anterior colporrhaphy were administered either placebo or prostaglandin E_2 in different doses (0.75 mg, 1.5 mg, or 2.25 mg) intravesically on postoperative days 6 and 7. Urodynamic assessment was performed before and after treatment. A moderate but not significant decrease of maximum bladder capacity, bladder compliance, and maximum urethral closure pressure was found in patients treated with 2.25 mg of prostaglandin E_2. These urodynamic changes did not correspond to the clinical outcome: Residual urine decreased and effective bladder capacity increased significantly in all four groups uninfluenced by the type of therapy. The rate of success (defined by the amounts of residual urine after therapy) was similar in the four groups. A long-term effect of prostaglandin E_2 could also be excluded, since the mean time interval from operation to the first day without residual urine was similar in the four groups. Therefore the therapeutic value of intravesically administered prostaglandin E_2 in doses from 0.75 to 2.25 mg must be seriously questioned. (AM J OBSTET GYNECOL 1985;151:375-9.)

Key words: Urinary retention, prostaglandin therapy

From clinical experience it is well known that partial or complete urinary retention presents a major and frequent problem after surgery of female incontinence. However, precise data about the frequency of this postoperative complication are rare and equivocal, ranging from 18%[1,2] to 35%.[3] To shorten this period of postoperative retention and to facilitate bladder emptying, different therapeutic modalities such as cholinergic or α-blocking drugs, intermittent catheterization, electrical stimulation are frequently used[4] but are often of minor efficacy. Preliminary reports indicated that intravesical application of prostaglandin E_2 seems to be efficacious for postoperative urinary retention.[5-8] Since most of these studies were uncontrolled, we compared in a randomized double-blind fashion the effect of prostaglandin E_2 in different dosages with placebo when administered intravesically. The aim of this study was to get a clear and objective answer regarding the clinical value of prostaglandin E_2 for the treatment of postoperative urinary retention. Nevertheless we were also interested in the characteristics of lower urinary tract function after prostaglandin E_2 application, and therefore urodynamic assessments were made before and after each treatment.

Material and methods

The criteria for selection of patients were complete urinary retention or partial retention with residual urine of >100 ml on postoperative day 5 after anterior colporrhaphy as primary treatment of urinary stress incontinence with or without hysterectomy. All patients were tested preoperatively for proof of urinary stress incontinence as follows: After filling the voided bladder until imperative urgency was present, the patient was asked to cough in supine and standing position. The

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Table I. Maximum bladder capacity (volume at imperative urgency) before and after treatment (ml, mean ± SD)*

	Pretherapeutic	*After first administration*	*After second administration*
Placebo	363.3 ± 94.8	366.7 ± 107.5	368.3 ± 53.4
0.75 mg prostaglandin E_2	310.0 ± 50.6	335.0 ± 48.9	331.7 ± 46.7
1.5 mg prostaglandin E_2	311.7 ± 100.7	343.3 ± 94.4	335.0 ± 95.9
2.25 mg prostaglandin E_2	290.0 ± 107.3	243.3 ± 27.3	268.3 ± 47.9

*Analysis of variance comparing the four groups: F = 2.4216 (NS). Analysis of variance comparing pretherapeutic and posttherapeutic values: F = 0.1025 (NS). Analysis of variance examining the interdependency of both variables: F = 0.6495 (NS).

result of this clinical incontinence test was evaluated as positive if synchronous leakage occurred which could be stopped by digital paraurethral elevation. This test was positive in all cases of the study. If, however, there was any discrepancy between the patient's history, the gynecologic examination, and the result of the clinical incontinence test, a urodynamic assessment (as described later) was done. No preoperative voiding disorders were found in the selected patients. Patients suffering from major cardiac, pulmonary, renal, or hepatic diseases or epilepsy were excluded from this study. Patients with micturition problems after other types of surgery for relief of incontinence or after radical hysterectomy were also excluded from this study. Immediately after operation an indwelling catheter was inserted and left in place until postoperative day 3. A microscopic and bacteriologic examination was performed before the onset of the study in all cases. No additional medication for urinary retention was given before and during the treatment period. All patients were informed about the experimental nature of this study and gave their fully informed oral consent.

Urodynamic assessment consisted of a cystometry and a urethral pressure profile measurement prior to the initiation of therapy and after each administration of the drug. Uroflowmetry was carried out when the patient was able to micturate. Cystometry was performed in the supine position with the use of saline solution at room temperature as the filling medium with a filling rate of 50 ml/min. As a provocative test the patient was asked to cough after every 100 ml of instilled fluid. The volumes at which the first desire to void and at which imperative urgency was present were recorded, the latter one being defined as maximum bladder capacity. Subsequently the urethral pressure profile measurement was performed again in the supine position. A twin-transducer catheter (Gaeltec Ltd.) and a balloon-rectal-catheter were connected with uroprofilemeter (Wiest Company, Berlin, Germany) for simultaneous registration of intravesical, intraurethral, and intrarectal pressure. All pressure values were recorded by a four-channel recorder and evaluated according to the usual criteria.[9, 10]

On postoperative day six patients fulfilling the above-mentioned criteria were given placebo or 0.75 mg, 1.5 mg, or 2.25 mg of prostaglandin E_2 after pretreatment urodynamic asessment. Coded ampules according to a randomization list were dissolved in 40 ml of saline solution at body temperature and instilled into the catheterized bladder. Patients were asked to avoid micturition until imperative urgency was present. If possible, the first micturition was recorded by uroflowmetry. Four to six hours after the intravesical administration a urodynamic assessment was carried out as described earlier. The ability to void spontaneously or a substantial reduction of residual urine after treatment was taken as a clinical criterion of success. Residual urine of >100 ml was always considered as therapeutic failure. On the next day (postoperative day 7) the same procedure was repeated with the use of the same coded solution as on day 6. In cases of unsuccessful treatment patients received identical stimulating therapy, which consisted of cholinergic drugs, electrostimulation, and intermittent catheterization starting on postoperative day 8. The time intervals to the absence of residual urine were evaluated as additional clinical criteria of success.

The code of the double-blind study was not opened until the last case was completed. According to the prospective randomized protocol 10 patients received placebo, six patients received 0.75 mg of prostaglandin E_2, 6 received 1.5 mg, and six received 2.25 mg. The patients' ages and the clinical pretherapeutic findings including bacteriuria did not differ substantially among the four groups. Statistical analysis of the clinical and urodynamic data were done by means of the χ^2 test and by multifactorial analysis of variance.

Results

Mean values of maximum bladder capacity were not significantly different in the four groups either before or after therapy (Table I). When values before and after therapy were compared in each individual group only treatment with 2.25 mg of prostaglandin E_2 resulted in a moderate but not significant decrease of maximum bladder capacity. Similar results were found when mean bladder compliance determined by filling cystometry were compared (Table II): Patients treated

Table II. Bladder compliance (ml/cm water, mean ± SD)*

	Pretherapeutic	*After first administration*	*After second administration*
Placebo	53.7 ± 30.1	44.5 ± 27.6	43.8 ± 24.5
0.75 mg prostaglandin E$_2$	46.7 ± 26.9	40.8 ± 22.7	37.5 ± 16.4
1.5 mg prostaglandin E$_2$	43.7 ± 32.3	67.0 ± 33.9	49.7 ± 31.6
2.25 mg prostaglandin E$_2$	50.0 ± 31.3	42.8 ± 28.9	32.6 ± 15.2

*Analysis of variance comparing the four groups: F = 0.2908 (NS). Analysis of variance comparing pretherapeutic and posttherapeutic values: F = 1.0549 (NS). Analysis of variance examining the interdependency of both variables: F = 1.1609 (NS).

Table III. Maximum urethral closure pressure (cm water, mean ± SD) with patient at rest in supine position*

	Pretherapeutic	*After first administration*	*After second administration*
Placebo	61.3 ± 21.3	63.7 ± 18.7	70.0 ± 16.1
0.75 mg prostaglandin E$_2$	62.7 ± 16.3	57.8 ± 21.6	65.0 ± 19.1
1.5 mg prostaglandin E$_2$	56.0 ± 9.7	61.3 ± 13.8	63.7 ± 23.7
2.25 mg prostaglandin E$_2$	56.3 ± 36.1	50.3 ± 28.1	43.0 ± 29.1

*Analysis of variance comparing the four groups: F = 0.6132 (NS). Analysis of variance comparing pretherapeutic and posttherapeutic values: F = 0.2413 (NS). Analysis of variance examining the interdependency of both variables: F = 1.5496 (NS).

Table IV. Residual urine, including amounts of 0 ml (ml, mean ± SD)*

	Pretherapeutic	*After first administration*	*After second administration*
Placebo:	331.7 ± 85.4	222.5 ± 163.5	195.0 ± 187.2
0.75 mg prostaglandin E$_2$	275.0 ± 90.5	280.0 ± 99.0	160.0 ± 144.1
1.5 mg prostaglandin E$_2$	263.3 ± 158.1	203.3 ± 158.5	180.0 ± 243.6
2.25 mg prostaglandin E$_2$	229.2 ± 78.5	225.0 ± 123.2	136.7 ± 80.4

*Analysis of variance comparing the four groups: F = 0.2370 (NS). Analysis of variance comparing pretherapeutic and posttherapeutic values: F = 6.2871 ($p < 0.01$). Analysis of variance examining the interdependency of both variables: F = 0.4742 (NS).

with the highest dose of prostaglandin E$_2$ demonstrated a marked decrease of bladder compliance, whereas changes were less consistent in the other groups. However, compliance values <20 ml/mbar, which are usually considered as abnormal indicating a hypertonic detrusor muscle or loss of bladder wall elasticity,[11] were found in only four of the total of 28 patients. Surprisingly, of these four patients each received a different amount of prostaglandin E$_2$, 0.75 mg, 1.5 mg, or 2.25 mg, or placebo. Therefore the decrease of bladder compliance observed after therapy seems to be of minor importance in regard to efficacy of prostaglandin E$_2$ treatment. Overactive detrusor function (unstable detrusor), defined as rapid increase of intravesical pressure with an amplitude of >15 cm of water controlled by simultaneous registration of intrarectal pressure, was a rare finding. Only two patients demonstrated such an abnormality, one after the first administration of 1.5 mg of prostaglandin E$_2$ and another after the second administration of 2.25 mg of prostaglandin E$_2$.

The results of maximum urethral closure pressure measurement in the supine position with the patient at rest are shown in Table III. Although at first sight these values seem high, one must take into account that these measurements were performed only 6 and 7 days after operation with paraurethral edema and infiltration probably present in some instances. This may also explain the great variability of the results. Whereas patients treated with placebo or 0.75 mg or 1.5 mg of prostaglandin E$_2$ showed a slight increase of urethral closure pressure, a clear decrease of these values could be observed in the high-dose group. These changes were, however, not statistically significant. Because of the frequent complete urinary retention, uroflowmetry was performed only in a small group of all patients. The applied therapy had no influence on maximal flow rate, which never exceeded 20 ml/sec.

Patients treated with 2.25 mg of prostaglandin E$_2$ complained about imperative urgency earlier than the other groups, but again these differences were not sta-

Table V. Effective bladder capacity, i.e., bladder capacity minus residual urine (ml, mean ± SD)*

	Pretherapeutic	*After first administration*	*After second administration*
Placebo	31.7 ± 56.7	144.2 ± 181.7	190.0 ± 212.8
0.75 mg prostaglandin E_2	35.0 ± 56.5	60.0 ± 80.0	171.7 ± 136.1
1.5 mg prostaglandin E_2	53.3 ± 82.6	140.0 ± 147.4	171.7 ± 146.1
2.25 mg prostaglandin E_2	61.7 ± 72.2	55.0 ± 89.4	136.7 ± 91.8

*Analysis of variance comparing the four groups: F = 0.2480 (NS). Analysis of variance comparing pretherapeutic and posttherapeutic values: F = 11.8338 ($p < 0.01$). Analysis of variance examining the interdependency of both variables: F = 0.7325 (NS).

Table VI. Clinical results of treatment*

	After first administration†			*After second administration‡*		
	No change	*Improved*	*Success*	*No change*	*Improved*	*Success*
Placebo	8	1	1	7	1	2
0.75 mg prostaglandin E_2	5	1	0	3	2	1
1.5 mg prostaglandin E_2	4	2	0	2	2	2
2.25 mg prostaglandin E_2	5	1	0	5	0	1

*No change, residual urine of >100 ml; improved, residual urine between 30 and 100 ml; success, residual urine of less than 30 ml.

†χ^2 = 3.1055 (NS).

‡χ^2 = 5.0620 (NS).

Table VII. Rate of complete urinary retention

	*Pretherapeutic**	*After first administration†*	*After second administration‡*
Placebo	6/10	5/10	5/10
0.75 mg prostaglandin E_2	3/6	3/6	2/6
1.5 mg prostaglandin E_2	2/6	2/6	1/6
2.25 mg prostaglandin E_2	2/6	1/6	0/6

*χ^2 = 1.5986 (NS).

†χ^2 = 2.1462 (NS).

‡χ^2 = 5.1333 (NS).

Table VIII. First postoperative day without residual urine*

	Mean ± SD
Placebo	12.7 ± 6.0
0.75 mg prostaglandin E_2	11.0 ± 3.2
1.5 mg prostaglandin E_2	10.0 ± 2.8
2.25 mg prostaglandin E_2	11.3 ± 2.7

*Analysis of variance comparing the four groups: F = 0.5257 (NS).

tistically significant. Discomfort like pain in the pelvis or bladder spasms were found in all groups, even in the placebo group. After the second administration half of the patients treated with the high dosage of prostaglandin E_2 complained about the described symptoms. Diarrhea was considered to be a possible side effect of prostaglandin therapy. This symptom was observed in only one patient, who after decoding was identified as having been treated with placebo.

Amounts of residual urine decreased (Table IV) and effective bladder capacity increased (Table V) during the treatment period. Both changes were statistically significant ($p < 0.01$) but uninfluenced by the type of therapy.

No significant correlation between the type of therapy and the prospectively defined parameters of success could be observed (Table VI). Although no complete urinary retention was present after the second administration of 2.25 mg of prostaglandin E_2, a similar decrease of the incidence of retention was also observed in the other treatment groups (Table VII). No long-term effect of therapy could be detected, since there was no significant difference in the time interval from operation to first day without residual urine (Table VIII).

Comment

In previous experimental studies a stimulating effect of prostaglandins on detrusor activity could be demonstrated not only in animal experiments[12, 13] but also in the isolated human bladder.[14] In contrast to prostaglandin $F_{2\alpha}$, which contracts all muscles of the lower urinary tract, prostaglandin E_2 has been shown to relax urethral smooth muscle.[15] Therefore this substance was considered a promising drug for treatment of urinary retention.[16] Indeed preliminary reports on local administration of prostaglandin E_2 were very enthusiastic[5-7, 17] or at least confirmatory.[8] More recently, however, growing concern about the usefulness of this type of therapy has emerged.[18, 19]

In an attempt to get clear and objective results we performed our study in a prospective randomized double-blind fashion (coding of the ampoules was performed by the Upjohn Company, Crawley GB). Since spontaneous remission of altered micturition becomes more frequent with increasing postoperative interval, we chose postoperative day 6 for the onset of treatment. The doses 0.75 and 1.5 mg of prostaglandin E_2 were similar to those in most previous uncontrolled trials.[6, 7] In addition, we included a group with the slightly higher dose of 2.25 mg of prostaglandin E_2. This latter dose led to a moderate decrease of maximum bladder capacity, bladder compliance, and urethral closure pressure. Half of these patients, however, complained about discomfort after prostaglandin instillation. The lack of any demonstrable overactive detrusor function (unstable detrusor) with the exception of two cases, which is in contrast to previous reports,[6] may be due to the fact that in this study cystometry was performed only 4 to 6 hours after administration of the drug.

Surprisingly, the clear though not significant urodynamic changes did not correlate at all with the clinical results: In all four groups a moderate decrease of residual urine and an increase of effective bladder capacity as well as an increase of the incidence of complete voiding could be observed after treatment. The rate of success in the placebo group points to the high incidence of spontaneous remission of altered micturition after vaginal repair and possibly to the importance of psychogenic factors in this complication.

The contrast between the clear urodynamic changes and the absence of a similar therapeutic response may be due to the short effect of intravesically administered prostaglandin E_2. In addition it has to be borne in mind that difficulties in micturition after vaginal repair may be caused by an inhomogeneous etiology. In summary, we cannot confirm the positive results of previous preliminary studies[5-8, 17] and therefore seriously question the clinical value of this type of therapy.

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C-reactive protein in pregnancy and in the postpartum period

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Serum C-reactive protein levels were measured by means of the nephelometric immunoassay technique in 215 pregnant women at various gestational ages. The results indicate a positive correlation of serum C-reactive protein levels with gestational age. In addition, we studied serum C-reactive protein concentrations in 37 pregnant women in labor and during the postpartum period. Delivery was accomplished by either the vaginal or abdominal route and was associated with significant C-reactive protein production. (AM J OBSTET GYNECOL 1985;151:380-3.)

Key words: C-reactive protein, pregnancy

The introduction of a new, sensitive method,[1] the nephelometric immunoassay, for the determination of C-reactive protein has spurred new interest in this active-phase protein. C-reactive protein is produced in the liver[2] and is present in normal individuals at concentrations ranging from 0.3 to 0.8 mg/dl.[1] C-reactive protein rises significantly following injury and inflammation.[3] Because of this latter association, it was thought that it could aid in the management of patients with preterm premature rupture of the membranes.[4]

There are conflicting reports as to the changes in C-reactive protein levels during pregnancy,[5-8] and no quantitative determinations have been conducted in the postpartum period. The fact that there are no established normal C-reactive protein levels in pregnancy and the postpartum period can be the reason for the contradictory opinions about the usefulness of C-reactive protein determinations as a predictor of infection in patients with premature rupture of membranes.[4, 9, 10]

The purpose of this study was to establish the normal values of C-reactive protein in a normal population during pregnancy and the postpartum period.

Material and methods

Two hundred fifty-two patients were studied at various gestational ages calculated from the last menstrual period and confirmed either by physical examination and/or follow-up by ultrasound examination.

A group of 215 patients not in labor and selected at random were sampled at various gestational ages from 8 to 42 weeks. Sampling for serum C-reactive protein was conducted during routine follow-up visits to the antenatal care clinic. At the time of sampling all the patients were healthy and had uncomplicated pregnancies and normal physical examination.

A second group of patients (N = 26) was selected at random when they entered the labor ward of Los Angeles County/USC Medical Center's Women's Hospital in labor; they had normal spontaneous vaginal deliveries and uneventful puerperiums.

A third study group selected at random included seven patients who had elective cesarean sections and four patients who underwent emergency cesarean section, the latter done at an early state of labor because of fetal distress. All of the cesarean section patients were administered prophylactic antibiotics for 24 hours following the procedure. All parturients were followed daily by body temperature, white blood cell count, and differential counts. The characteristics of the study groups are summarized in Table I.

Measurements of serum C-reactive protein levels were performed by a nephelometric immunoassay with the Beckman's immunochemistry analyzer, with use of monospecific goat C-reactive protein antiserum and C-reactive protein control serum (supplied by Beckman Instruments Inc., Fullerton, CA). The sensitivity of the assay is 0.2 mg/dl. The within-assay and between-assay coefficient of variation was 2.6 and 7.4, respectively. The data were plotted and statistical differences were tested with a linear regression analysis or calculated by means of a Student's t test, Wilcoxon sign rank test, and rank sum test as appropriate. Bonferroni procedure was applied to account for the multiple testing (Figs. 2-4).

Results

The results are illustrated in Figs. 1 to 4. Fig. 1 shows serum C-reactive protein levels at various gestational ages. A linear regression analysis indicates a positive

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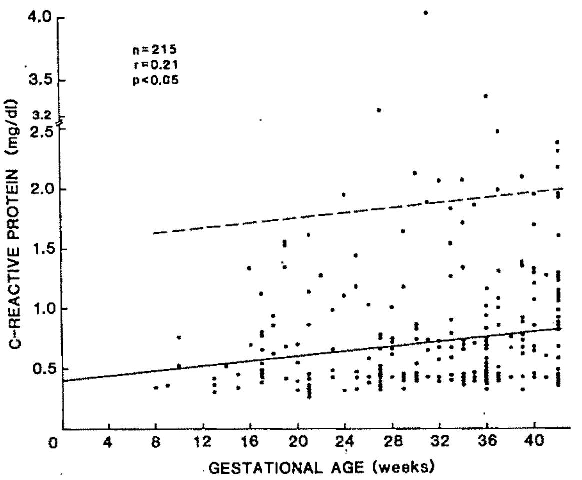

Fig. 1. C-reactive protein levels throughout pregnancy. The *solid line* represents the predicted mean value of C-reactive protein for a given gestational age. The *broken line* represents the 95% confidence limit on the predicted individual C-reactive protein value for a given gestational age.

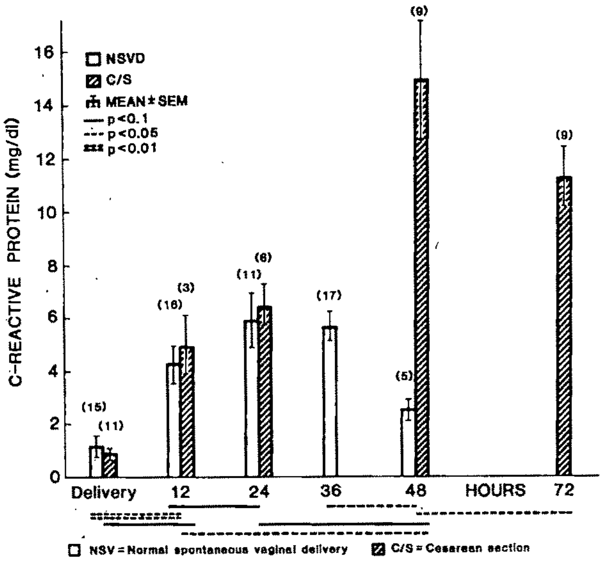

Fig. 2. C-reactive protein levels at delivery and during postpartum period.

Table I. Characteristics of the study groups

Group	*n*	*Age (yr)*	*Parity*	*Gestational age (wk)*
Pregnancy	215	23.4 ± 4.8	1.5 ± 7.1	8-42
Normal spontaneous vaginal delivery	26	23.4 ± 4.8	1.0 ± 1.0	40.3 ± 1.6
Cesarean section	11	30.7 ± 5.5	2.5 ± 3.2	39.3 ± 2.4

correlation that is statistically significant at $p < 0.05$ for these two variables ($r = 0.21$).

Delivery, either by vaginal or abdominal route, was associated with significant C-reactive protein production. The mean C-reactive protein value for the vaginal deliveries rose from 1.19 mg/dl to 5.98 mg/dl 24 hours later and declined to 2.51 mg/dl 48 hours after delivery. The changes after cesarean section delivery were even greater, with elevations from 0.78 mg/dl to 14.92 mg/dl at 48 hours, declining to 11.25 mg/dl at 72 hours post partum (Fig. 2). The changes in the other monitored parameters are illustrated in Figs. 3 and 4. The peak levels of white blood cell counts and bands precede those of C-reactive protein by 12 to 24 hours in the groups of patients delivered either vaginally or abdominally.

The minimal decrease in the postpartum hematocrit level had a marginal effect on the serum C-reactive protein levels. There was no significant change in body temperature during the study period.

Comment

Normal values for C-reactive protein in pregnancy have not yet been established. The majority of previous reports are based on the semiquantitative latex agglutination or similar techniques,[5-8] and demonstrate an increased presence of C-reactive protein with progress in gestation. In disagreement with these reports, Connell and Connell[7] observed no change and Yeh[8] reported finding declining values throughout pregnancy.

Only Hawrylyshyn et al.[10] report normal values obtained by the nephelometric technique. Contrary to our findings, in their study, C-reactive protein levels did not correlate with gestational age. This discrepancy could be attributed to the fact that in their study the control group was too small ($n = 74$) to reach statistical significance. Although in our study the linear correlation is significant ($p < 0.05$), the low r-value (0.21) implicates only 4% (r2) of the variability that can be related to the gestational age.

Indirect confirmation of our findings comes from the study of Evans et al.[4] In their study the cut-off point for the upper normal levels of C-reactive protein was 2 mg/dl. This is in agreement with our data for 95% confidence limit on predicted individual value for C-reactive protein, 1.81 to 1.91 mg/dl, at 26 to 36 weeks' gestational age (Fig. 1), which for practical purposes is the same as the data of Evans et al.

The cause for the rising concentration of C-reactive protein during pregnancy is not yet known. Two pos-

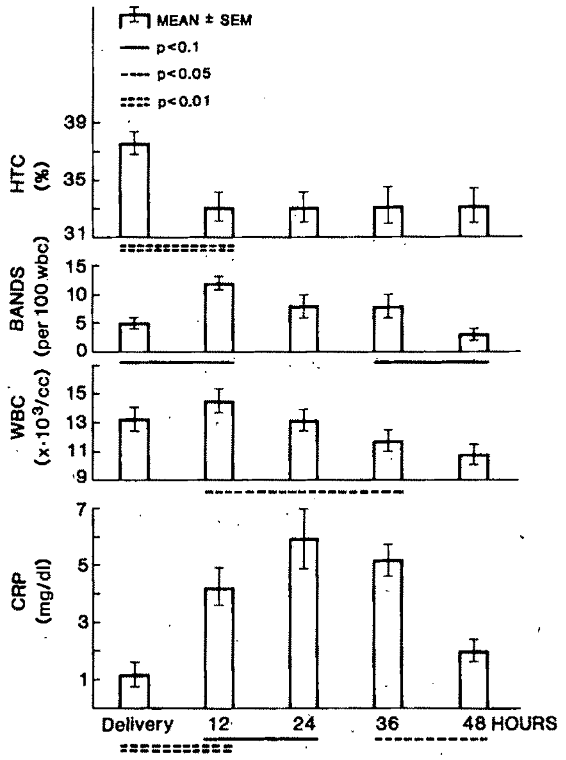

Fig. 3. C-reactive protein *(CRP)*, white blood cell count *(WBC)*, band cell count, and hematocrit *(HTC)* levels at delivery and post partum in patients with normal spontaneous vaginal delivery.

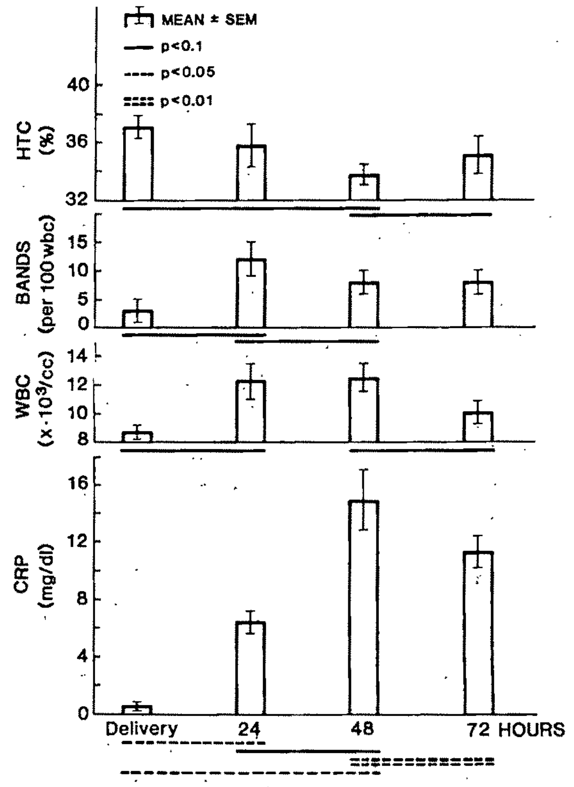

Fig. 4. C-reactive protein *(CRP)*, white blood cell count *(WBC)*, band cell count, and hematocrit *(HTC)* levels at delivery and post partum in patients with delivery by cesarean section.

sible explanations can be derived from the literature. The prevailing explanation for elevated C-reactive protein concentration is that it is a response to an inflammatory/necrotic process. We can speculate that the placenta in going through the process of aging, as well as possible microscopic lesions of varying degrees, could also stimulate production of C-reactive protein. If that is the case, the expected rising levels should be much higher than the ones actually found. C-reactive protein is the most sensitive among the acute-phase proteins, increasing thousandfolds in association with inflammation and/or necrosis.[3]

A much more plausible explanation is suggested by the studies of Connell and Connell[7] and Schumacher.[11] These studies found an increased incidence in detection of C-reactive protein in women using contraceptive pills that contain estrogens and progestogens, as compared to the control group. It is probable that C-reactive protein reflects rising estrogen levels and, to a lesser degree, progestational agents.

Independent of the rise during pregnancy, increased levels observed after cesarean sections could be attributed to cellular injury. Such changes have been reported to occur following abdominal surgical procedures.[12] The same mechanism of C-reactive protein production may follow the normal spontaneous vaginal delivery. Furthermore, the difference between abdominal and vaginal delivery in C-reactive protein surge can be attributed to various degrees of tissue injury. In a study by Schumacher[13] similar quantitative differences of serum C-reactive protein levels were related to the degree of tissue traumatization during abdominal hysterectomy compared to that during vaginal repair.

Another possible link to elevated C-reactive protein could be the rise in prostaglandins during labor.[14] In animal and human studies, prostaglandins were reported to stimulate an increase in acute-phase serum proteins.[15, 16]

Our data demonstrate a consistent postpartum surge in C-reactive protein, similar to that described by Fisher et al.[12] Recognizing such constant patterns is of diagnostic aid when trying to determine the significance of any further increases, e.g., infection.

The physiologic changes that occur in labor and puerperium could interfere in the diagnostic process, in which we normally observe an increase in white blood cell count, band count, or erythrocyte sedimentation rate. Two C-reactive protein determinations, obtained at 24 and 48 hours post partum or 48 and 72 hours after abdominal delivery, can be useful in diagnosing developing infection.

We thank Barbara Davis for her excellent technical assistance.

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Transient ventricular dysfunction associated with cesarean section in a patient with hyperthyroidism

Steven L. Clark, M.D., Jeffrey P. Phelan, M.D., Martin Montoro, M.D., and Jorge Mestman, M.D.
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Pulmonary artery catheterization was performed prior to surgery in a severely hyperthyroid patient undergoing cesarean section. A transient but significant decline in left ventricular performance was observed in conjunction with the stress of operation. A parallel is suggested between this phenomenon and the documented exercise-induced reduction of left ventricular function in nonpregnant hyperthyroid patients. (Am J Obstet Gynecol 1985;151:384-6.)

Key words: Pregnancy, heart, ventricular dysfunction, hyperthyroidism

Cardiac function is generally enhanced in the presence of hyperthyroidism. Recent noninvasive studies, however, have suggested the occurrence of a transient, exercise-induced depression of myocardial performance in the presence of thyrotoxicosis. The present case provides additional evidence of this phenomenon derived from invasive cardiovascular studies and suggests management considerations in patients with uncontrolled hyperthyroidism who undergo operative delivery.

Case report

A. A., a 21-year-old primigravid woman, was admitted to Women's Hospital in preterm labor. In addition to uterine contractions, she complained of fatigue, palpitations, and heat intolerance of several weeks' duration. There was no history of cardiac disease.

The pulse was 120 bpm; blood pressure, 110/70 mg Hg; and oral temperature, 98.0° F. Physical examination revealed signs of hyperthyroidism. Mental status was normal. Cardiac auscultation was unremarkable, and there was no evidence of cardiac or pulmonary disease.

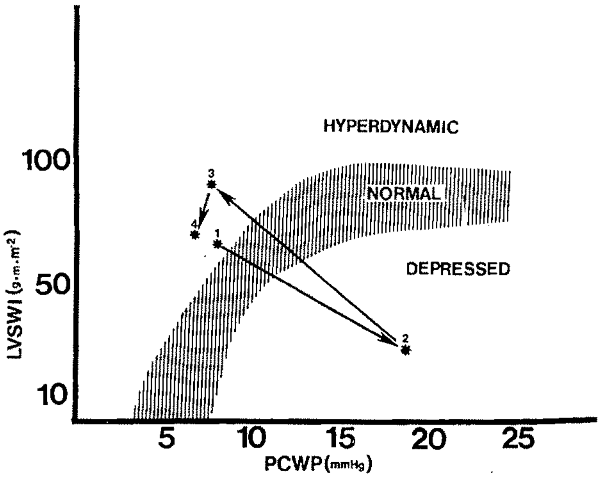

Fig. 1. Left ventricular function in a patient with thyrotoxicosis. *1*, Before operation; *2*, ½ hour after operation; *3*, 1½ hour after operation; *4*, 24 hours after operation. Curve modified from: Ross J Jr, Braunwald E. The study of left ventricular function in man by increasing resistance to ventricular ejection with angiotensin. Circulation 1964;29:739.

Initial thyroid function tests were: triiodothyronine uptake, 1.14 μg/dl (normal, 0.88 to 1.19 μg/dl); thyroxine, 26.1 μg/dl (normal, 4.5 to 13.2 μg/dl); free thyroxine index, 29.8 μg/dl (normal, 4.5 to 13.2 μg/dl), triiodothyronine, 454 ng/dl (normal, 70 to 275 μg/dl); and free triiodothyronine index, 518 μg/dl (normal, 70 to 275 μg/dl). Real-time ultrasound revealed a single fetus in the breech position with measurements consistent with a 28-week gestation. Regular uterine contractions were noted on the external tocodynamometer and the fetal heart rate was 168 bpm. On the basis of clinical findings, the diagnosis of hyperthyroidism was made and the patient was begun on a regimen of propylthiouracil and propranolol, 20 mg orally every 6 hours. Magnesium sulfate tocolysis was initially successful. Thirty-six hours after admission the patient again began to experience regular uterine contractions and an elevation of temperature to 102° F. The uterus was moderately tender to palpation and the fetal heart rate was 176 bpm. Amniocentesis revealed 5 to 10 white blood cells per high-power field with numerous motile bacilli. The diagnosis was a 28-week intrauterine pregnancy with breech presentation, amnionitis, and hyperthyroidism.

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Table I. Hemodynamic indices in a patient with hyperthyroidism

Time	*Blood pressure (mm Hg)*	*Pulse (BPM)*	*Mean pulmonary artery pressure (mm Hg)*	*Pulmonary capillary wedge pressure (mm Hg)*	*Central venous pressure (mm Hg)*	*Cardiac output (L/min)*	*Left ventricular stroke work index ($g \cdot m \cdot m^{-2}$)**
1	150/70	110	23	8	6	9.0	66
2	170/70	140	41	18	6	4.2	23
3	165/69	93	21	7	5	10.5	96
4	149/80	102	21	6	4	7.7	75

Time: 1 = Before operation; 2 = ½ hour after operation; 3 = 1½ hour after operation; 4 = 24 hours after operation. Normal mean pulmonary artery pressure, 10 to 17 mm Hg; normal pulmonary capillary wedge pressure, 5 to 12 mm Hg; normal central venous pressure, 5 to 12 mm Hg; normal cardiac output, 5 to 6 L/min.

*Based on body surface area of 1.5 m^2.

In anticipation of cesarean section, a flow-directed pulmonary artery catheter and an arterial line were placed, and the patient received 1 gm of sodium iodide intravenously. Initial hemodynamic measurements are detailed in Table I. A chest x-ray film obtained just before operation showed no evidence of pulmonary edema. A low vertical cesarean section was performed with the patient under general anesthesia with the use of a balanced nitrous oxide/oxygen technique. A 1310 gm infant was delivered with Apgar scores of 2 and 7. During operation the patient received 750 ml of lactated Ringer's solution. Estimated blood loss was 500 ml. During operation, the pulmonary artery pressure rose to a maximum of 44/15 mm Hg. One half hour after the operation the patient appeared agitated and diaphoretic. Temperature was 98.8° F. Bibasilar rates were apparent on chest auscultation. Physical examination and hemodynamic indices (Table I) were consistent with congestive heart failure. She received propranolol, 0.5 mg by slow intravenous push during 3 minutes, and furosemide, 20 mg intravenously. The patient responded in the course of the next hour with a diuresis of 1000 ml, a fall in pulmonary capillary wedge pressure, and an increase in cardiac output. The patient remained stable and the pulmonary artery catheter was removed 24 hours later, with final readings as detailed in Table I. Calculated left ventricular stroke work indices are plotted against wedge pressure in Fig. 1.

Comment

In patients with thyrotoxicosis, the stress of acute infection or operation may precipitate clinical deterioration, including, in extreme cases, thyroid storm. Such patients are extremely poor operative risks because the stress of operation itself may further aggravate an already unstable clinical condition.

Hyperdynamic circulation and enhanced myocardial performance in hyperthyroid patients are well documented. However, numerous studies have demonstrated the development of cardiac hypertrophy and congestive heart failure in animals with prolonged experimental hyperthyroidism, as well as the development of papillary muscle dysfunction and mitral regurgitation in humans.[1]

Recent studies by means of radionuclide ventriculography have demonstrated an exercise-induced reduction in left ventricular ejection fraction in hyperthyroid patients.[2] Pretreatment with propranolol did not influence the exercise-induced reduction in left ventricular ejection fraction, thus suggesting a reversible catecholamine-independent cardiomyopathy. We provide here further direct evidence of a reversible decline in left ventricular performance associated with the acute stress of operation in a patient with thyrotoxicosis. Examination of Fig. 1 reveals the left ventricular stroke work index to be in the hyperdynamic range before operation. In the intraoperative and immediate postoperative periods the patient experienced a transient but dramatic decline in left ventricular performance that resolved during the next hour. A striking parallel is suggested between this patient, who also received pretreatment with propranolol, and the aforementioned patients with transient exercise-induced reduction in left ventricular ejection fraction. A similar peripartum decline in left ventricular performance associated with a high-output state has been noted in patients with severe preeclampsia.

The use of a β-blocking agent in a patient with left ventricular failure is, in general, contraindicated. In this case, however, the patient's clinical condition, including an abrupt rise in systolic pressure and pulse, suggested impending thyroid storm. Because of the serious consequences of thyroid storm and in view of the patient's hyperdynamic presurgical myocardial performance, it was elected to cautiously administer a small dose of propranolol.

The deleterious fetal effects of untreated hyperthyroidism in pregnancy are well recognized. This case illustrates the potential for major short-term intrapartum maternal morbidity, even in thyrotoxic patients who are not experiencing thyroid storm, and emphasizes the need for prompt recognition and treatment of hyperthyroidism in pregnancy. In normal pregnant patients, cardiac output increases up to 65% in the immediate postpartum period because of an "autotransfusion" associated with removal of the products of conception and contraction of the uterus. For patients with conditions causing relatively fixed cardiac output (for instance, mitral stenosis), the limited ability to accommodate this relatively increased intravascular volume is well known. Patients with a stress-induced depression of left ventricular performance associated with hyperthyroidism may, in a similar manner, be unable to accommodate this relative increase in volume and may develop pulmonary edema.

The clinician should be alert to this unusual but potentially serious problem when dealing with any thyrotoxic patient subjected to operation. For such patients intensive monitoring of left heart function and careful fluid restriction would seem indicated.

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BASIC SCIENCE SECTION

Prostaglandin production and stimulation by angiotensin II in the isolated perfused human placental cotyledon

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Levels of prostaglandins E and $F_{2\alpha}$, thromboxane B_2, and 6-oxo-prostaglandin $F_{1\alpha}$ were measured by radioimmunoassay in the maternal and fetal effluents of isolated human placental cotyledons perfused in vitro. All prostaglandins measured were released in greater amounts by the maternal side than by the fetal side of the perfused cotyledon although there were no consistent concentration gradients between the two sides. The approximate rank order of prostaglandin release into the maternal side was thromboxane $B_2 >$ prostaglandin $F_{2\alpha} \simeq$ prostaglandin E $\simeq$ 6-oxo-prostaglandin $F_{1\alpha}$, and that into the fetal side was thromboxane $B_2 \simeq$ prostaglandin $F_{2\alpha} \simeq$ prostaglandin E $\simeq$ 6-oxo-prostaglandin $F_{1\alpha}$. Injection of angiotensin II (0.5 μg) into the fetal circulation stimulated prostaglandin E and 6-oxo-prostaglandin $F_{1\alpha}$ but not thromboxane B_2 and prostaglandin $F_{2\alpha}$ release into the fetal circulation and had no effect on maternal release. Angiotensin II (0.5 μg) had no effect on either side of the perfused cotyledon when injected into the maternal circulation. It is proposed that prostaglandin release into both maternal and fetal circulations may be flow-dependent and that the angiotensin II–stimulated release of prostaglandin E and 6-oxo-prostaglandin $F_{1\alpha}$ may serve to modulate the vasoactive actions of angiotensin II on the fetal vasculature.

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Key words: Placenta, prostaglandin production, angiotensin II

There has been much speculation concerning the role of prostaglandins released by the placenta. It has been suggested that prostaglandin E_2 (PGE_2) may regulate inequalities of maternofetal perfusion by acting as a vasoconstrictor of the fetal-placental vessels and as a vasodilator of the uterine vasculature.[1] Prostacyclin (PGI_2) of placental origin may contribute to the elevated plasma PGI_2 levels and the refractoriness to angiotensin II (AII) found in pregnancy.[2] It may also safeguard maternal blood supply to the placenta by preventing platelet aggregation although this process may also be influenced by placental thromboxane A_2 (TxA_2) synthesis since TxA_2 is a potent platelet aggregator.

Prostaglandin production by the fetal-placental vasculature could be involved in controlling blood flow through these vessels since it has been shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 contract the fetal vessels of the placenta whereas PGI_2 relaxes them.[3] Release of prostaglandins into the fetal circulation may also give rise to systemic effects within the fetus contributing to the low vascular resistance of the fetal circulation[4] and the patency of the ductus arteriosus.[5]

In pregnant rabbits, AII plays an important part in the maintenance of systemic blood pressure,[6] and the same may be the case in the human since plasma AII levels correlate with diastolic blood pressure in pregnant women.[7] AII may play an important role in the maintenance of uterine blood flow which is seen to decrease when the conversion of angiotensin I (AI) to AII is blocked by captopril, an inhibitor of angiotensin-converting enzyme. Control of uterine blood flow may involve PGE_2 since AII increases the production of PGE_2 in the pregnant uterus[6, 8] although the exact site of production within the uteroplacental complex is unclear. We have previously shown that components of the renin-angiotensin system act as very potent vasoconstrictors of the fetal-placental vasculature of the isolated perfused human placental cotyledon and it was proposed that, in vivo, the renin-angiotensin system may participate in the control of fetal-placental blood flow.[9] Prostaglandins may modulate the actions of this system in the fetal-placental vasculature. Meclofena-

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mate, an inhibitor of prostaglandin synthesis, decreases the contractile response of fetal-placental vessels to AII, suggesting that prostaglandins normally potentiate the contractile response.[3] To examine further the possible role of prostaglandins in the control of placental blood flows, we have studied the release of prostaglandin E (PGE), $PGF_{2\alpha}$, 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$, the stable metabolite of PGI_2), and thromboxane B_2 (TxB_2, the stable metabolite of TxA_2) from both sides of the isolated perfused human placental cotyledon preparation and looked at the modification of this production by AII administered into either the fetal or the maternal circulation.

Method

Placentas from women with uncomplicated pregnancies were collected within 5 minutes of vaginal delivery or cesarean section at term.

Placental cotyledons, free of infarcts or tears, were perfused by a method which has been described in detail elsewhere.[9] Briefly, this involved perfusing tissue culture medium 199 (Gibco) containing polyvinylpyrrolidone (5% w/v) (Sigma Chemical Co.) through a cannulated chorionic artery on the fetal surface of the placenta and collecting the effluent from the associated chorionic vein. Perfusion of the intervillous space was achieved by the insertion of two intravenous 21-gauge butterfly needles through the maternal surface of the perfused cotyledon. The whole preparation was maintained at 37° C in a controlled temperature cabinet. The flow rates of the fetal and maternal perfusates were maintained at 4 and 10 $ml \cdot min^{-1}$, respectively. The resulting perfusion pressures from both sides were between 30 and 60 mm Hg.

In a preliminary series of experiments (three placentas) fractions of fetal and maternal effluent (1.5 ml) were collected at 20, 30, 40, 50, and 60 minutes after the establishment of the maternal circulation and immediately placed on ice at 4° C. Medium flowing through the perfusion tubing without the cotyledon in place was collected to serve as a blank. The samples were centrifuged (1700 × g) immediately after each experiment to remove any blood contamination and the supernatants (1 ml) stored at $-20°$ C for subsequent extraction and analysis of prostaglandins. The samples were extracted with the use of Sep-Pak C_{18} cartridges (Waters) prior to radioimmunoassay. This removed interference from polyvinylpyrrolidone which was evident when direct radioimmunoassay of perfusates was used. The cartridges were first wetted with 20 ml of methanol/water (4:1, v/v) and residual methanol removed by washing with deionized/distilled water (20 ml). The perfusate sample (1 ml) was acidified to pH 3.0 with formic acid, loaded on the cartridge, and eluted with diethyl ether (10 ml), and the fractions were collected in two siliconized glass test tubes. The solvent was evaporated under nitrogen and the residue resuspended in phosphate-buffered saline (Dulbecco) (1 ml).

The extracted samples were assayed for prostaglandins with radioimmunoassays developed in this laboratory for PGE,[10] $PGF_{2\alpha}$,[10] 6-oxo-$PGF_{1\alpha}$,[11] and TxB_2. The TxB_2 antisera cross reacted <1% with 6-oxo-$PGF_{1\alpha}$, prostaglandin E_1, PGE_2, $PGF_{2\alpha}$, prostaglandin A_2, and arachidonic acid and <5% with prostaglandin D_2.

The preliminary experiments revealed a large amount of variability in the results and that the levels of prostaglandins were sometimes very close to the level of detection of the assays. The experiments were repeated (three placentas) with collection of larger fractions of the fetal and maternal effluents (5 ml on the fetal side and 15 ml on the maternal side). Samples were treated and extracted in the same way as before, except that 4 ml of the fetal perfusate and 10 ml of the maternal perfusate were extracted and resuspended in 2 ml of phosphate-buffered saline, which would theoretically concentrate the prostaglandins two and five times, respectively. The samples were assayed as before for PGE, $PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$, and TxB_2.

To assess the effect of AII on the production of prostaglandins from the perfused cotyledon, control samples (5-minute duration of flow) were collected from both sides of three placentas beginning 25 minutes after the establishment of maternal perfusion. AII (Sigma Chemical Co., 0.5 μg in 0.5 ml of gassed perfusate) was injected into the maternal circulation and fetal effluent collected for a further 5 minutes. Further control samples of maternal and fetal effluent were taken (5 minutes' duration) 10 minutes after baseline perfusion pressures had been reestablished. A second dose of AII (0.5 μg in 0.5 ml) was then injected into the fetal circulation and fetal and maternal effluents collected for 5 minutes. All medium collected was treated and extracted as described above. The residue was resuspended in phosphate-buffered saline (2 ml) and assayed for $PGF_{2\alpha}$, PGE, 6-oxo-$PGF_{1\alpha}$, and TxB_2.

All results of prostaglandin assays were expressed as concentration of the prostaglandin per milliliter of medium after correction for any blank reading obtained. Using labeled prostaglandins, we found that recoveries for all prostaglandins were approximately equal (range, 82% to 94%) and thus no corrections were made to the prostaglandin levels obtained after the extraction procedure. As the PGE antisera could not distinguish PGE_1 and PGE_2, results from this assay are taken to indicate total E series prostaglandin, expressed in this paper as PGE.

Statistical significance was calculated by Student's *t* test.

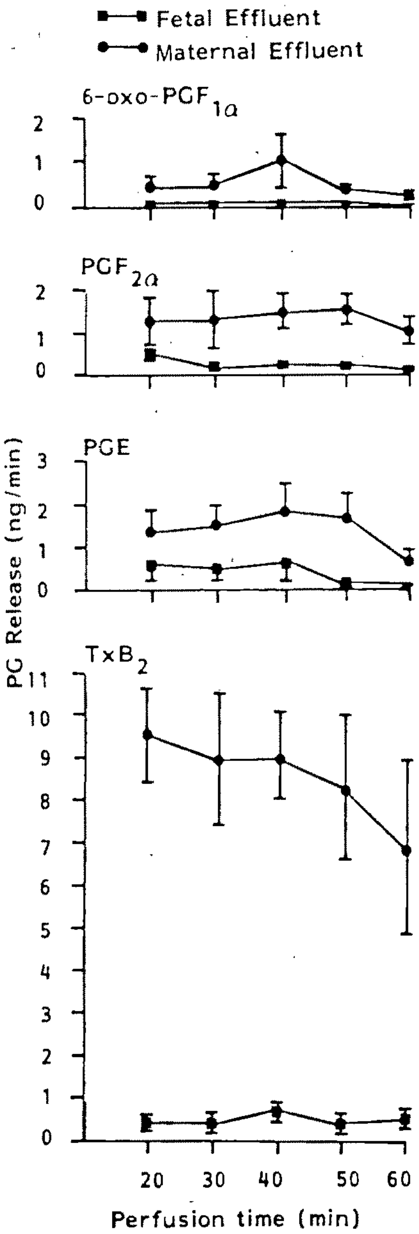

Fig. 1. Prostaglandin release into fetal (■——■) and maternal (●——●) circulations of the isolated perfused human placental cotyledon during 60 minutes of perfusion (results are mean ± SEM of six experiments).

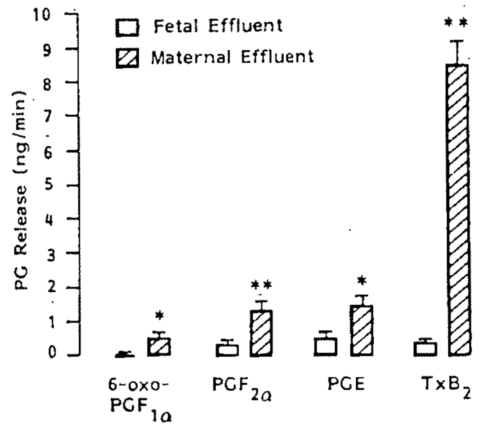

Fig. 2. Mean prostaglandin release during 60 minutes of perfusion into fetal *(open columns)* and maternal *(hatched columns)* circulations of the isolated perfused human placental cotyledon (results are mean ± SEM of six experiments. Significant difference from fetal circulation: * = $p < 0.01$ and ** = $p < 0.005$).

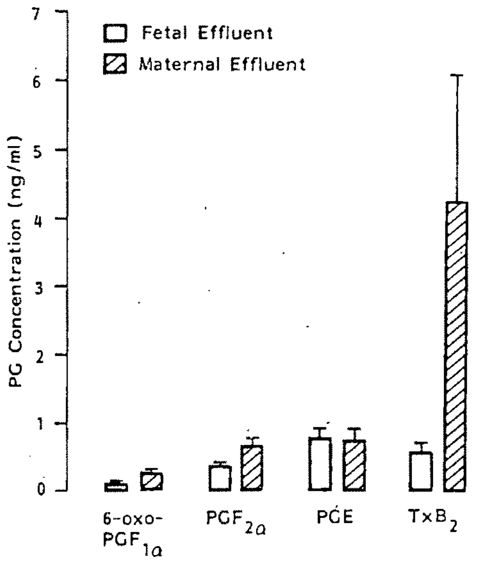

Fig. 3. Mean prostaglandin concentration during 60 minutes of perfusion in fetal *(open columns)* and maternal *(hatched columns)* circulations of the isolated perfused human placental cotyledon (results are mean ± SEM of six experiments).

Results

None of the prostaglandins measured, apart from TxB_2, showed any consistent trend in the amount released from either side of the placenta during the 60 minutes of perfusion (Fig. 1). TxB_2 release showed a steady decline on the maternal side which tended to parallel the gradual washout of blood from the intervillous space. When the means of all values obtained at each time point throughout the course of perfusion were calculated, no statistical differences were found between the mean amounts of prostaglandin released in the preliminary experiments and the repeat experiments, and so the two sets of results were combined (Fig. 2). The amount of each prostaglandin released into the maternal circulation was significantly greater than the amount released into the fetal circulation (TxB_2, $p < 0.0005$; $PGF_{2\alpha}$, $p < 0.005$; PGE, $p < 0.01$; and 6-oxo-$PGF_{1\alpha}$, $p < 0.01$). When the results were expressed in terms of concentrations of prostaglandins per milliliter of perfusate (Fig. 3) no difference was

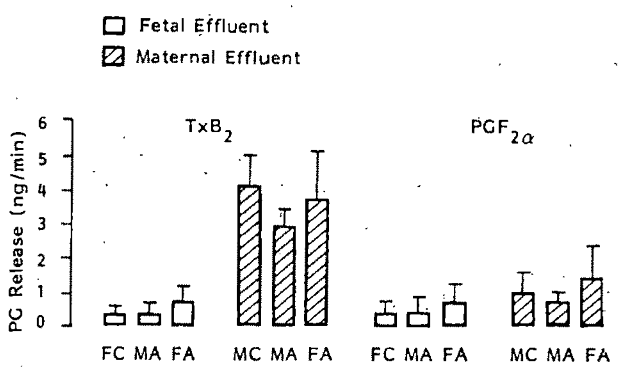

Fig. 4. TxB_2 and $PGF_{2\alpha}$ release into fetal *(open columns)* and maternal *(hatched columns)* circulations before (*FC* and *MC*) and after injection of AII (0.5 μg) into the fetal *(FA)* and maternal *(MA)* circulations of the isolated perfused human placental cotyledon (results are mean ± SEM of three experiments).

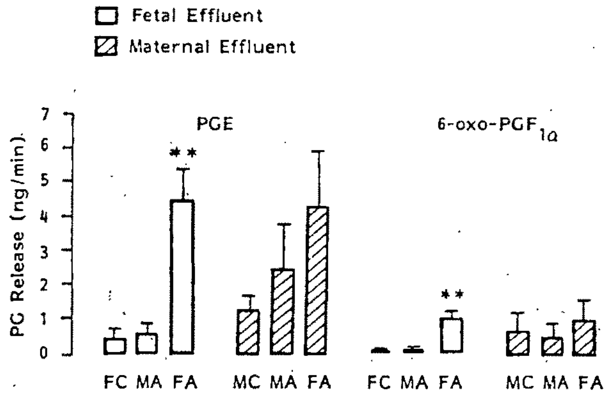

Fig. 5. PGE and $6\text{-oxo-}PGF_{1\alpha}$ release into the fetal *(open columns)* and maternal *(hatched columns)* circulations before (*FC* and *MC*) and after injection of AII (0.5 μg) into the fetal *(FA)* and maternal *(MA)* circulations of the isolated perfused human placental cotyledon (results are mean ± SEM of three experiments. Significant difference from fetal control: ** = $p < 0.005$).

seen between the two sides for any of the prostaglandins.

The amounts of prostaglandins released into the maternal circulation had an approximate rank order of $TxB_2 > PGF_{2\alpha} \simeq PGE \simeq 6\text{-oxo-}PGF_{1\alpha}$, and those released into the fetal circulation had a rank order of $TxB_2 \simeq PGE \simeq PGF_{2\alpha} \simeq 6\text{-oxo-}PGF_{1\alpha}$. After injection of AII into the fetal circulation the rank orders of release were $TxB_2 \simeq PGE > PGF_{2\alpha} \simeq 6\text{-oxo-}PGF_{1\alpha}$ for the maternal side and $PGE > TxB_2 \simeq PGF_{2\alpha} \simeq 6\text{-oxo-}PGF_{1\alpha}$ for the fetal side.

Injection of AII into either the maternal or the fetal circulation did not alter TxB_2 or $PGF_{2\alpha}$ release on either side of the placenta (Fig. 4) when compared with the control preinjection levels (the control samples taken before injection of AII into the fetal and maternal sides showed no statistical differences and so they were combined). However, injection of AII into the fetal circulation significantly increased the amount of PGE ($p < 0.005$) and $6\text{-oxo-}PGF_{1\alpha}$ ($p < 0.005$) (Fig. 5) released into the fetal perfusate but did not significantly increase the release into the maternal perfusate. PGE and $6\text{-oxo-}PGF_{1\alpha}$ levels were not significantly affected by injection of AII into the maternal circulation of the placenta. The amounts of PGE and $6\text{-oxo-}PGF_{1\alpha}$ released into the fetal circulation by AII stimulation were found to be significantly greater (PGE, $p < 0.01$; $6\text{-oxo-}PGF_{1\alpha}$, $p < 0.01$) than the average amounts released during the time-course experiments and so were not the result of variability of the preparation.

The increase in fetal perfusion pressure which resulted from the injection of AII into the fetal side was 16 ± 3 mm Hg, and when AII was injected into the maternal side the resulting increase in fetal perfusion pressure was 3 ± 1 mm Hg. These pressure increments are in agreement with our previously reported data.[9]

Comment

The use of the isolated perfused human placental cotyledon preparation for the study of prostaglandin release offers many distinct advantages over conventional techniques such as tissue culture or superfusion of tissue and tissue slices, the main one being that production can be assessed from both sides of the placenta in a dynamic and more physiologic approximation of in vivo conditions. With this system it was found that there was a greater release of all prostaglandins measured into the maternal circulation than into the fetal circulation (Fig. 2) although there were no consistent concentration gradients between the two sides (Fig. 3). One exception to this was that the concentration of TxB_2 in the maternal effluent was generally much higher than its concentration in the fetal effluent but this changed with time. The release of TxB_2 on the maternal side tended to parallel the progressive washout of blood elements from the intervillous space, including platelets, which are known to produce large amounts of TxA_2. Thus the high levels of TxB_2 found in the maternal effluent probably reflect platelet rather than placental production.

Prostaglandins are highly lipid-soluble and so they would be expected to cross the placenta with relative ease. The amounts of prostaglandin released into either side of the placenta would thus reflect local tissue production and release directly into the perfusate and also the relative amounts being transferred from one circulation into the other. This transfer would in turn depend on the concentration gradient and the flow rates on either side of the placenta. As no consistent concentration gradients were observed for the prostaglandins across the placenta, the fact that significantly more prostaglandin was released into the maternal circulation than into the fetal circulation was probably due to the greater flow rate on the maternal side and suggests that in this preparation the release of prostaglandin into either side may be flow-dependent. If this is the case in vivo, one consequence of this would be that if the flow was reduced on the maternal side more prostaglandins would be released into the fetal circulation where they could act to reduce blood flow and thus preserve the maternal-fetal perfusion ratio. In this context, it is interesting to note that in chronically catheterized sheep, embolization of the uterine vascular bed with microspheres produced parallel decreases in umbilical blood flow.[12]

In this study, prostaglandin release showed a rank order (except for TxB_2 into the maternal circulation) of $TxB_2 \simeq PGE \simeq PGF_{2\alpha} \simeq$ 6-oxo-$PGF_{1\alpha}$. This compares with the results reported by Mitchell[13] for superfused placentas where the approximate rank order was $TxB_2 \simeq PGE > PGF_2 \simeq$ 6-oxo-$PGF_{1\alpha}$ and by Dembele-Duchesne et al.[14] for placental homogenates where the order was $TxB_2 \sim PGE_2 \simeq$ 6-oxo-$PGF_{1\alpha}$. This contrasted with the production of prostaglandins after incubation of the placental homogenates with endoperoxide (PGH_2) where PGE_2 was the predominant prostaglandin produced.[14] Caution should be expressed when one is interpreting the relative importance of placental production of one prostaglandin over another as it can be seen that the relative amounts of prostaglandin produced change with the preparation used. Also the expression of the relative rank order of production does not take into account the differences in the biologic activity of the various prostaglandins.

In our preparation, the pattern of prostaglandin production changed after injection of AII into the fetal circulation, which stimulated PGE and 6-oxo-$PGF_{1\alpha}$ but not TxB_2 and $PGF_{2\alpha}$ release into the effluent. AII has been shown to stimulate PGE release from the pregnant uterus,[8] kidney,[15] and lung[16] and thereby to modulate the pressor action of AII. PGI_2, a potent vasodilator, may act in a similar manner since AII has been shown to stimulate PGI_2 release from fetal rabbit lungs.[17] Thus the release of PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$) and PGE from the fetal vasculature in response to AII may serve as an important regulatory process maintaining the fetal-placental blood flow in the face of increased fetal peripheral resistance due to circulating AII. AII failed to stimulate prostaglandin release significantly into the maternal effluent, and this suggests that the release of prostaglandins into the two circulations may have certain independent control mechanisms. The absence of maternal release of prostaglandins in response to stimulation by AII also suggests that the observed AII-induced increase of PGE_2 release from the pregnant uterus of other animals may not be of placental origin.

AII increases fetal perfusion pressure when injected into the maternal circulation. This response is probably not mediated by any of the prostaglandins measured in this study since AII had no effect on the amounts released into the fetal circulation when it was injected on the maternal side.

Work is now in progress in this laboratory to examine the effects of endogenously produced and exogenously added prostaglandins with or without AI or AII on the fetal vasculature of this system. This work will expand on the interactions of prostaglandins and AII reported here.

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β-Adrenergic receptors in human myometrium during pregnancy: Changes in the number of receptors after β-mimetic treatment

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The concentration of available β-adrenoceptors has been studied in the myometrium of women treated with terbutaline for premature uterine contractions and in an untreated control group. Myometrial strips were taken at cesarean section from the lower uterine segment and the uterine fundus. The concentration of β-adrenoceptors was determined with a radioligand binding assay. In untreated women we found no difference in the concentration of β-adrenoceptors in the uterine fundus compared to that in the lower uterine segment. The cyclic adenosine monophosphate production after β-adrenoceptor agonist stimulation in vitro was equal in both locations. In the terbutaline-treated women, the binding site concentrations in both the fundus and lower uterine segment were lower compared to those in the control group. The decrease was most pronounced in the fundus where receptor concentration was only half that found in the control group. The results suggest that treatment with β-mimetics causes a desensitization in the β-adrenoceptor system of human myometrium during pregnancy. This desensitization may partly explain the limited duration of the relaxant effect of β-mimetics often seen in the treatment of preterm labor. (AM J OBSTET GYNECOL 1985;151:392-6.)

Key words: Preterm labor, β-adrenergic receptors, terbutaline, cyclic adenosine monophosphate

The efficiency of β-adrenoceptor–stimulating drugs such as terbutaline in inhibiting premature uterine contractions for shorter periods is well documented.[1,2] The effect of this treatment in prolonging pregnancy for longer periods is, however, disputed.[3]

In previous in vitro studies on human myometrium during pregnancy we have found a direct correlation between increased cyclic adenosine monophosphate production after β-mimetic stimulation and smooth muscle relaxation.[4] The cyclic adenosine monophosphate increase, however, was reduced after about 30 minutes in spite of continuous active agonist stimulation. The decrease in cyclic adenosine monophosphate production was followed by a return of spontaneous contractions in the preparations tested. Thus these experiments showed a rapid β-adrenoceptor desensitization in vitro. Myometrial biopsy specimens from women treated with terbutaline for at least 2 weeks and in whom labor could not be arrested showed a lower basal cyclic adenosine monophosphate content as well as a lack in response to in vitro β-adrenoceptor stimulation when compared to untreated control subjects.[5] These results could partly be explained by the increased activity of the enzyme phosphodiesterase found in the myometrium of the terbutaline-treated women.[5]

In rat myometrium a reduction in the concentration of β-adrenoceptors was found by Johansson and Andersson[6] after in vivo isoprenaline or terbutaline treatment.

In the present study we have examined the β-adrenoceptors in myometrium from pregnant women either untreated or treated with terbutaline during the last weeks of pregnancy because of premature uterine contractions. The women were delivered by cesarean section for obstetric reasons, myometrial biopsies were taken, and the concentration of β-adrenoceptors were determined with the radioligand technique described by Hayashida et al.[7]

Material and methods

Myometrial biopsies from the lower uterine segment and uterine fundus were taken from women delivered by cesarean section for obstetric reasons. These specimens were dissected free of endometrium and serosa. The biopsies were taken before any oxytocic drug had been administered. One group of women (n = 8) had been treated with terbutaline. Untreated women (n = 11), delivered in the thirty-eighth to forty-third week of gestation, served as a control group. For more

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Table I. Women treated with terbutaline before cesarean section

Patient No.	*Duration of treatment*	*Amount of terbutaline administered*	*In labor*	*Biopsy from*		*Indication for cesarean section and gestational age*
				Isthmus	*Fundus*	
1	25 days	500 mg orally	No	Yes	Yes	Disproportion/breech, 38 wk
2	50 days	750 mg orally	Yes	Yes	Yes	Placenta previa, 36 wk
3	50 days	600 mg orally	No	Yes	No	Disproportion, 38 wk
4	60 days	900 mg orally	No	Yes	No	Disproportion/twins, 37 wk
5	16 hr	5 mg intravenously	Yes	Yes	No	Disproportion, 35 wk
6	1 hr	0.5 mg intravenously	Yes	Yes	Yes	Asphyxia, 40 wk
7	20 days	400 mg orally	No	Yes	No	Humanitarian, 37 wk
8	20 days	300 mg orally	No	Yes	No	Growth retardation, 32 wk

detailed information concerning the terbutaline-treated women, see Table I.

The biopsies were kept in Ringer's solution and analyzed within 30 minutes of operation or stored at −70° C until later analysis.

Determination of cyclic adenosine monophosphate content. The myometrial biopsies from the lower uterine segment and uterine fundus of the untreated women were stimulated with increasing concentrations (1.5 to 15 μmol/L) of terbutaline. The samples were then rapidly frozen in a mixture of freon plus solid carbon dioxide at −70° C. The frozen samples were homogenized in 5% trichloroacetic acid and the nucleotides were separated on columns of AG-1-X8 (200 to 400 mesh, formate form). After elution with formic acid the eluates were lyophilized and thereafter redissolved in 50 mmol/L of sodium acetate, pH 4.5, before analysis. The analysis was performed essentially as described by Gilman.[8]

Assay of tritium-labeled dihydroalprenolol binding to myometrial membrane preparations. The membrane preparations obtained from the untreated and terbutaline-treated women were assayed for tritium-labeled dihydroalprenolol binding according to Johansson and Andersson.[6] The membrane suspensions (300 to 500 mg of protein) were incubated with various concentrations of tritium-labeled dihydroalprenolol (0.5 to 12 nmol/L; specific activity, 90 to 120 Ci/mmol) in an incubation buffer (50 mmol/L of Tris hydrochloride, pH 7.5; 10 mmol/L of magnesium hydrochloride) in the absence or presence of 30 μmol/L of propranolol in a total volume of 0.18 ml. After incubation for 30 minutes at 37° C, the reaction was stopped by rapid dilution of the assay mixture with 3 ml of ice-cold incubation buffer. This was followed by rapid vacuum filtration through a Whatman glass microfiber filter (GF/C) with the use of a Millipore sampling manifold. The filters were then rapidly washed four times with 3 ml of the ice-cold incubation buffer. After drying, radioactivity bound to the filters was determined. Specific binding to β-adrenoceptors was defined as the difference between the amount of radioactivity bound in the presence of tritium-labeled dihydroalprenolol and the nonspecific binding seen in the presence of tritium-labeled dihydroalprenolol plus 30 μmol/L of propranolol. In our experiments the specific binding was about 50%.

The rank order of potency of agonists competing for tritium-labeled dihydroalprenolol binding sites was determined in the untreated group. Tritium-labeled dihydroalprenolol was incubated with myometrial membranes in the presence of increasing concentrations of agonists or antagonists. Binding was expressed as a percentage of specific binding in the absence of agonist or antagonist (Fig. 1). The inhibition constant (K_I) was determined for the adrenergic agonists as described by Cheng and Prusoff.[9] Protein concentration was determined according to Lowry et al.[10]

Statistical methods. All values are given as mean values ± SEM. In all experiments the results were statistically calculated with Student's *t* test on unpaired samples.

This study was approved by the Ethical Committee of Linköping University, Linköping, Sweden.

Results

Cyclic adenosine monophosphate activity. The cyclic adenosine monophosphate activity in myometrium from women previously untreated with β-adrenergic drugs during pregnancy is described in Fig. 2. The basal cyclic adenosine monophosphate content in myometrium from the lower uterine segment was comparable to that found in the uterine fundus. After stimulation in vitro with increasing concentrations of terbutaline the cyclic adenosine monophosphate production in both locations was increased. No significant differences in the concentrations of cyclic adenosine monophosphate produced by the lower

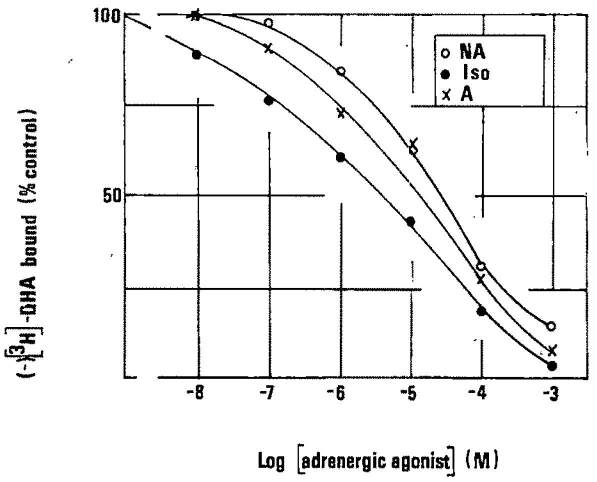

Fig. 1. Rank order of potency of agonists competing for tritium-labeled dihydroalprenolol binding sites in myometrial preparations from untreated women. The K_I values for the agonists were: isoprenaline *(Iso)* = 0.8 μmol/L; epinephrine *(A)* = 4.2 μmol/L, and norepinephrine *(NA)* = 6.5 μmol/L.

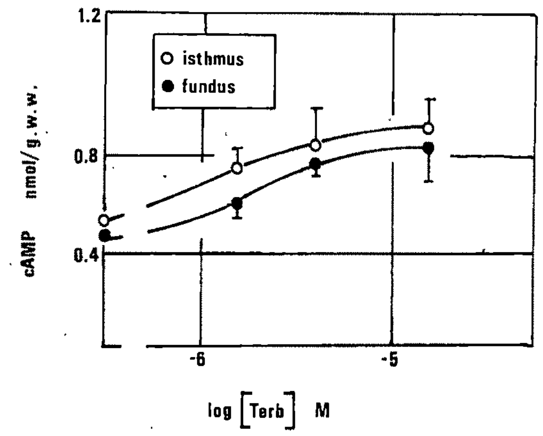

Fig. 2. Cyclic adenosine monophosphate *(cAMP)* production in myometrial preparations from the lower uterine segment (isthmus) and uterine fundus of untreated women after in vitro terbutaline *(Terb)* stimulation (mean values ± SEM).

Table II. Effects of terbutaline treatment on the number and affinity of β-adrenergic receptors in the uterine fundus and lower uterine segment of pregnant women

	No. of tritium-labeled dihydroalprenolol binding sites (fmol/mg protein)	*K_d of tritium-labeled dihydroalprenolol binding (nmol/L)*
Fundus		
Control (n = 6)	38.9 ± 1.2	5.8 ± 1.2
Terbutaline (n = 3)	21.1 ± 0.8*	6.1 ± 1.4
Lower uterine segment		
Control (n = 11)	37.4 ± 2.5	6.9 ± 0.6
Terbutaline (n = 8)	27.3 ± 1.4†	5.3 ± 1.7

K_d = Equilibrium dissociation constant.
*$p<0.001$.
‡$p<0.005$.

uterine segment and the uterine fundus were found (Fig. 2).

Tritium-labeled dihydroalprenolol binding. By testing various β-adrenoceptor–agonist acting substances for their potency to compete with the binding of tritium-labeled dihydroalprenolol to myometrial membranes, the following order of potency was found: (−)isoprenaline > (−)epinephrine > (−)norepinephrine (Fig. 1). Competition for binding was stereospecific for propranolol. Myometrial biopsies from the lower uterine segment and the uterine fundus of untreated women were studied as described above in order to determine the concentration of binding sites expressed as femtomoles per milligram of protein. The concentration of binding sites was similar in the uterine fundus (38.9 fmol/mg of protein) and in the lower uterine segment (37.4 fmol/mg of protein) (Fig. 3, Table II).

Binding sites in the lower uterine segment from untreated and terbutaline-treated women differed with a significantly lower concentration of tritium-labeled dihydroalprenolol binding sites in the terbutaline-treated group as shown in Fig. 4, *A*. A Scatchard plot (Fig. 5) of experiments dealing with the binding of tritium-labeled dihydroalprenolol showed that the concentrations of β-adrenoceptors were 27.3 fmol/mg of protein in the terbutaline-treated group and 37.4 fmol/mg of protein in the untreated group (Table II). In myometrial preparations from the uterine fundus there was an almost 50% decrease in binding capacity in preparations from terbutaline-treated women (21.1 fmol/mg of protein) compared to those from the untreated control group (38.9 fmol/mg of protein) (Fig. 4, *B*) (Table II).

The affinity for the receptors was comparable in all

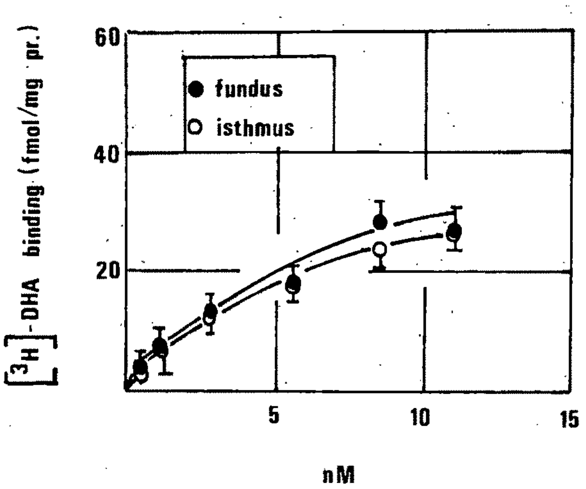

Fig. 3. Number of β-adrenoceptor binding sites in the lower uterine segment (isthmus) and uterine fundus of untreated pregnant women (mean values ± SEM).

studied preparations as the equilibrium dissociation constant (K_d) of tritium-labeled dihydroalprenolol was almost equal in all preparations (Table II).

Comment

The mechanism behind the desensitization of the β-adrenoceptor system has been studied by many investigators using different species and tissues.[11] Clinically, a reduced response to β-mimetic treatment has been noticed in the treatment of asthmatic patients[12] and in the treatment of premature uterine contractions.[13] Different mechanisms behind the reduced β-adrenoceptor response have been postulated.[14]

The desensitization may be agonist-specific, that is, after agonist stimulation, the number of receptors may be reduced or an uncoupling between receptor and effector may take place. Furthermore, the desensitization may depend on, for example, a decrease of the total adenylate cyclase activity or an increased destruction of cyclic adenosine monophosphate by the enzyme phosphodiesterase.[11]

There is experimental evidence that all of these mechanisms may operate to cause desensitization in the β-adrenoceptor system. In earlier studies we found a reduction in the concentration of β-adrenoceptors in the rat uterus[6] and an increase in phosphodiesterase activity in both rat[6] and human[5] uterine tissue after adrenoceptor stimulation.

In the present investigation we studied the distribution of β-adrenoceptors in different parts of the pregnant human uterus as well as changes in the concentration of receptors after in vivo β-adrenergic stimulation with terbutaline.

Using a radioligand binding technique, we found a comparable concentration of β-adrenoceptors in the uterine fundus and the lower uterine segment in normal pregnant women (Fig. 3). This is in accordance with the cyclic adenosine monophosphate levels found in the two locations after in vitro terbutaline stimulation (Fig. 2).

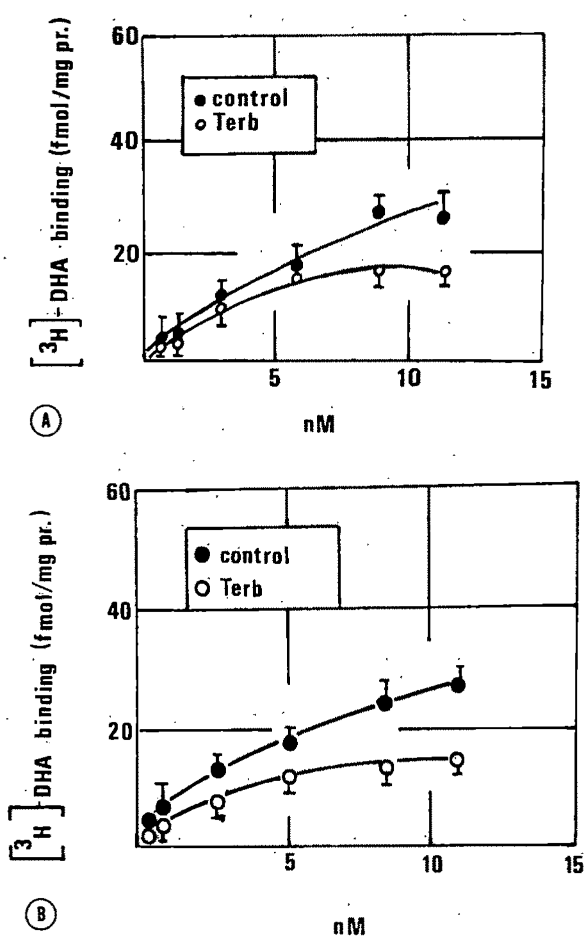

Fig. 4. Number of β-adrenoceptor binding sites in the lower uterine segment *(A)* and the uterine fundus *(B)* of terbutaline-treated women *(Terb)* and untreated women *(control)* (mean values ± SEM).

By assessing the order of effectiveness of adrenergic drugs in competing for binding sites with tritium-labeled dihydroalprenolol, we found that the adrenergic agonists competed in a manner compatible with β-pharmacologic potencies (Fig. 1); which is in accordance with Hayashida et al.[7] In our study, however, norepinephrine was more potent than reported by Hayashida et al. Whether this is due to different methods or different myometrial material is impossible to determine since it is not stated in their study in what trimester of pregnancy the biopsy was performed.

In women treated with terbutaline during pregnancy, a reduction in the number of β-adrenoceptors in the lower uterine segment was found. The reduction was most pronounced in the uterine fundus where an al-

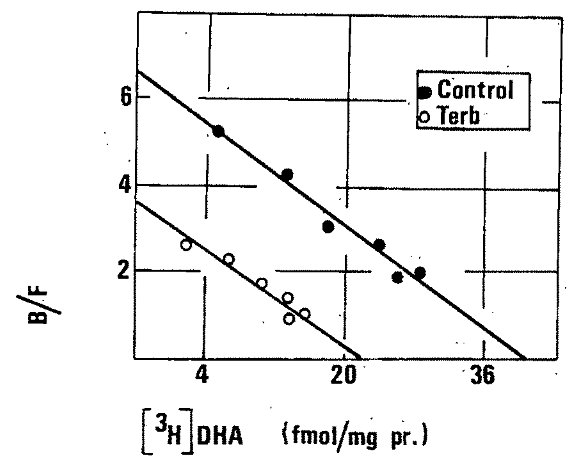

Fig. 5. Effects of terbutaline *(Terb)* on the specific binding of tritium-labeled dihydroalprenolol to myometrial membranes. The representative binding data from one untreated *(Control)* woman and one terbutaline *(Terb)*–treated woman is presented in the form of a Scatchard plot.

most 50% reduction in binding sites was seen. This loss of receptor sites is comparable with that found after in vivo β-adrenoceptor stimulation in rat myometrium by Johansson and Andersson[6] and in human leukocytes by Galant et al.[15] Why the reduction in receptor sites is more pronounced in the uterine fundus is difficult to explain. The number of fundal biopsy specimens studied was low. However, different distributions of the drug terbutaline might explain part of the discrepancy in desensitization in the uterine fundus and the lower uterine segment.

The concentration of β-adrenoceptors in the present study was low, possibly because of the fact that we have studied myometrium from pregnant women in the third trimester. In binding assays on myometrium from nonpregnant women, we have found a three times higher concentration of β-adrenoceptors than in pregnant human myometrium (unpublished data).

The reduction in receptor concentration mentioned above could be due to a tight binding of the agonist to the receptor, or the receptors may become internalized or lost in some other way. There is no known way to reduce this loss in receptor capacity other than the spontaneous recovery in time. Another factor, the increase in phosphodiesterase activity during treatment with betamimetics,[5] seems also to be involved in the desensitizing process and this effect might be counteracted by a phosphodiesterase-inhibiting drug. In recent studies on human myometrium we have shown that papaverine has a potent phosphodiesterase-inhibiting effect.[16] Thus combined tocolytic therapy with a β-adrenoceptor stimulant, for instance, terbutaline, and a phosphodiesterase-inhibiting drug, for instance, papaverine, could constitute a new approach to the improved treatment of preterm labor.

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Increase in cervical extensibility during labor induced after isolation of the cervix from the uterus in pregnant ewes

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Surgical transection of the cervix was done on eight ewes in late pregnancy. This procedure resulted in most of the length of the cervix being mechanically disconnected from the uterus. Labor was induced by injection of dexamethasone phosphate into a fetal hind limb during operation in four of the eight ewes. The success of the induction of labor was confirmed by observation of increased uterine activity and by measurement of the concentrations of progesterone and 17β-estradiol in peripheral plasma. Ewes were put to death approximately 48 hours after injection of dexamethasone and the extensibility of the isolated portion of the cervix was determined. Cervices taken from ewes in which labor had been induced were found to have softened considerably compared to control cervices taken from ewes in which the cervix had been transected without induction of labor. The results suggest that cervical softening can still occur during labor in the absence of any direct mechanical or local vascular connection between the cervix and uterus. (AM J OBSTET GYNECOL 1985;151:397-402.)

Key words: Uterine activity, cervical dilatation, ovine parturition

Parturition cannot be achieved without adequate uterine contractions and concurrent cervical softening and dilatation. In pregnant sheep, the endocrine changes leading to the generation of uterine contractions have been investigated extensively. The parts that are played by changes in placental enzyme activities and enhanced uterine production of prostaglandins have been studied in depth. The mechanism by which the structure of the cervix is remodeled to permit delivery, a change no less dramatic than the onset of uterine contractions, has received less attention. It has long been held that cervical softening is produced by the forces imposed on it by the contractions of the uterus. It has become apparent more recently, however, that cervical softening is the result of biochemical changes in the connective tissue of the cervical matrix[1] and that these changes can occur in the absence of uterine contractions.[2-4] It would therefore appear that cervical softening and uterine contractility represent two separate aspects of the process of parturition, both of which must be coordinated to achieve a successful delivery.

Our aim in the present study was to determine whether the cervix was capable of undergoing the biochemical changes leading to softening after being physically separated from the uterus. By this means we hoped to learn whether the forces of uterine contractions or any local vascular or neural connections between uterus and cervix were necessary for cervical softening to take place. Physical separation by cervical transection in vivo provided a means by which these connections could be disrupted while leaving the cervix with a systemic vascular supply from the vaginal arteries. Initiation of labor by intrafetal injection of dexamethasone during operation allowed the effects of labor on the transected portion of the cervix to be determined.

Parturition in the sheep is preceded by a considerable reduction in the concentration of progesterone in peripheral plasma, followed by a rise in the concentration of 17β-estradiol.[5, 6] Similar changes occur in the blood supply to the cervix.[7] The cervix should continue to receive these hormonal signals following transection, and it was thought likely that any alteration in the mechanical properties of the transected cervix during labor could be ascribed to a response of the cervical tissue to these changes in steroid hormone concentrations. The preparation used in this study therefore allowed us to observe the behavior of the uterus and the cervix in labor, while preventing any local communication between the two systems.

This study used a mechanical test of the extensibility of a strip of cervical tissue measured in vitro as a means of assessing the effect of our experimental manipulations on the cervix. Uterine activity was monitored continuously from the time of operation to ensure the success of the induction of labor. Labor was induced by using an intrafetal injection of the synthetic glucocorticoid dexamethasone because this is a rapid and re-

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producible means of achieving powerful uterine contractions. Concentrations of 17β-estradiol and progesterone were measured in plasma collected from blood samples taken at intervals from the jugular vein. These results were used to confirm that the changes in steroid hormone concentration seen during normal labor were still taking place in our animals.

Material and methods

Transections were conducted on eight Suffolk crossbred ewes between days 116 and 130 of pregnancy. All ewes carried singleton pregnancies. General anesthesia was induced by intravenous injection of thiopental sodium (Pentothal, Abbott Laboratories Ltd., Kent, United Kingdom) and maintained following tracheal intubation with 2% to 4% halothane in oxygen, administered by using a closed-circuit system. Carbon dioxide was absorbed by using soda-lime. A maternal jugular vein and the amniotic cavity were catheterized with polyvinyl chloride catheters (inside diameter of 2 mm; outside diameter of 3 mm; Portex Ltd., Hythe, Kent, United Kingdom) according to conventional aseptic procedures. In ewes 1 through 4, a fetal hind limb was delivered through an incision in the uterine wall. The fetus was injected with 40 mg of dexamethasone phosphate (Decadron, Merck, Sharp & Dohme Ltd., Hoddesdon, Herts, United Kingdom), containing 33.3 mg of dexamethasone. Injections were made into the fetal thigh and leg muscles in seven sites, as described by Flint et al.[8] The fetuses of ewes 5 through 8 received similar multiple injections of sterile saline (9 gm/L).

The cervix was exposed by extending the original paramidline abdominal incision and retracting the mammary gland. After identification of the uterocervical junction, the superficial parametrial tissues overlying the vascular plexus surrounding the cervical connective tissue were reflected at a point approximately 3 cm from the junction with the uterus. Each vessel in the plexus at this point was then undermined by blunt dissection, ligated twice, and cut between these sutures. This procedure allowed access to the cervix, which was then transected. Since the major vessels had been ligated immediately above and below this point, there was very little blood loss. Complete hemostasis was achieved by using electrocoagulation. Mucus was identified in the exposed cervical lumen that was then closed with discs 1.5 cm in diameter of sterile silicone rubber (Silastic, Dow Corning Corporation, Midland, Michigan). These were attached with cyanoacrylate ester adhesive (Loktite Products, Welwyn Garden City, Herts, United Kingdom). The area was packed with cellulose gauze (Oxycel, Parke-Davis Co. Pontypool, Monmouth, United Kingdom), and the abdomen closed.

Postoperatively the ewes received Streptapen (4 ml) containing penicillin G procaine (250 mg/ml) and dihydrostreptomycin sulfate (250 mg/ml) (Glaxovet Ltd., Greenford, Middlesex, United Kingdom) intramuscularly. This treatment was repeated on the following day. The ewes were also given pethidine (100 mg) intramuscularly. The ewes were kept in metabolism cages and fed 1 kg of dried grass nuts and 1 kg of ewe and lamb pencils with free access to water. Catheters were flushed with sterile saline (9 gm/L) containing 250 U/ml of heparin. Recordings of amniotic fluid pressure changes were made continuously by using a type 4-442 physiologic pressure transducer (Bell & Howell Ltd., Basingstoke, United Kingdom) and conventional amplifying and recording equipment (Devices Ltd., Welwyn Garden City, Herts, United Kingdom). Blood samples were drawn into chilled heparinized tubes containing 0.1 ml of aspirin, 0.5 mg/ml, and 0.1 ml of ethylenediaminetetraacetic acid, 7 mg/ml of blood, and centrifuged immediately for 15 minutes at 900 g and 4° C. Plasma samples were stored at −20° C until analysis.

Assays. Progesterone was measured in plasma by radioimmunoassay as described previously.[8] Assay reliability criteria did not differ significantly from those described previously. The interassay coefficient of variation, calculated from standards containing 1 ng of progesterone per milliliter of plasma included in each assay, was 12.5%; the intra-assay coefficient of variation, determined by assay of 12 aliquots of plasma each containing 100 pg of progesterone, was 9.1%. Concentrations of 17β-estradiol in plasma were measured by using an adaptation of the method of Flint et al.,[8] (with antiserum donated by Dr. B. J. A. Furr, I.C.I. Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, United Kingdom). Plasma blanks obtained from ovariectomized ewes and plasma standards containing known amounts of 17β-estradiol were included in each assay. Throughout the period during which the samples described in this paper were assayed, the interassay coefficient of variation was 9% and the intra-assay coefficient of variation, determined by assay of 12 aliquots of plasma each containing 100 pg of 17β-estradiol, was 3.1%. Extraction recoveries were 74.6 ± 4.3% (mean ± 1 SD; $n = 5$). Results were corrected for recovery. Sensitivity of the assay, defined as the mass of 17β-estradiol detected 2 SD from the lowest point on the standard curve, was 5 pg per tube. In specificity studies, cross-reactivities calculated as described by Abraham[9] were: for androstanedione, cortisol, 17α-hydroxyprogesterone, progesterone, and testosterone, <0.1%; for estrone, 2.22%; for estriol, 0.29%; and for 17α-estradiol, 0.25%.

Postmortem studies. Ewes were stunned by a captive bolt and were killed by exsanguination. Ewes 1 through 4, in which labor had been induced, were killed 48 to 50 hours after injection of dexamethasone. Ewes 5 through 8 were killed 49 to 90 hours after injection of saline solution. At the end of each experiment the uterus was opened and the fetus examined in situ. The

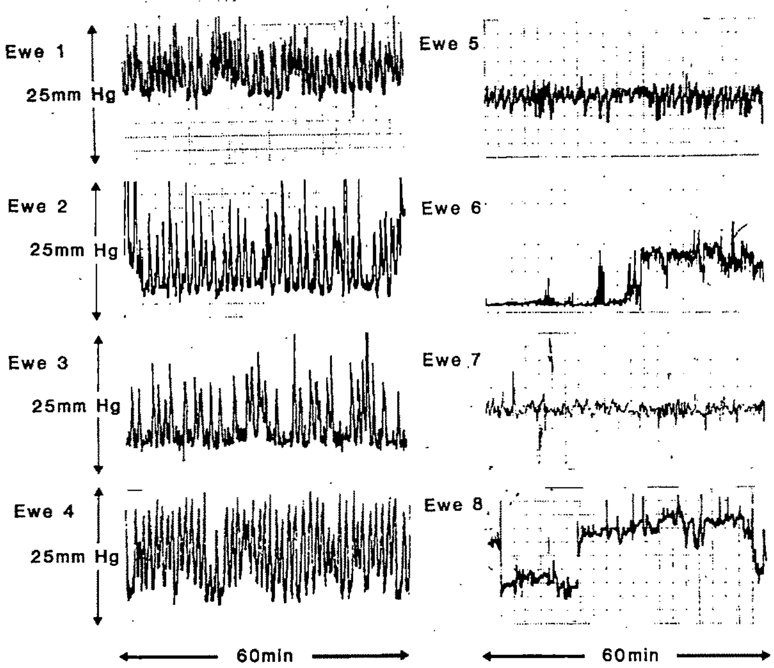

Fig. 1. Recordings of changes in amniotic fluid pressure occurring after intrafetal injection of dexamethasone (ewes 1 through 4) or sterile normal saline solution (ewes 5 through 8). Each recording is a specimen made during the hour immediately before sacrifice of each animal.

cervix and lower uterine segment were detached and taken on ice to the laboratory. Each cervix was then trimmed of parametrial tissue and opened longitudinally to reveal the internal structure of the cervical canal. A transverse section 15 mm wide, including the central cervical ring, was then cut. This strip, composed of the entire width of the cervix, was always taken from the distal transected portion of cervix that had been separated from the uterus at operation and always lay at least 1 cm from the site of transection.

Extensibility was measured by using an apparatus adapted from that originally described by Harkness and Harkness.[10] This equipment has been described previously for use with the cervix of the ewe.[4]

Results

Intrafetal injection with dexamethasone has been shown to lead to delivery between 40 and 70 hours later.[8, 11] Amniotic fluid pressure recordings begun approximately 3 hours after injection of dexamethasone already showed evidence of uterine activity, with contractions of 10 to 15 mm Hg in amplitude occurring approximately every 20 minutes. The frequency and amplitude of contractions increased to produce almost continuous trains of contractions some 30 hours later. Fig. 1 shows specimen recordings taken from each of the eight ewes. Each tracing was recorded during the hour immediately before sacrifice. Intrafetal injection of saline solution did not produce contractions of this type. Infrequent, small-amplitude contractions were observed for approximately 12 hours after operation. These subsequently subsided so that little activity was apparent.

Concentrations of progesterone and 17β-estradiol in maternal jugular venous plasma followed the pattern established for these hormones after induction of labor[8] in that there was a fall in the concentration of progesterone (Fig. 2) with a subsequent rise in the concentration of 17β-estradiol (Fig. 3). Hormone levels in plasma collected from the control ewes did not follow this pattern and showed little variation during the period of observation.

Postmortem examination. Examination of the transected cervices immediately after sacrifice revealed no visible necrosis of either portion other than that of the cut ends themselves, which were blackened following electrocoagulation. The vessels of the cervical plexus that had been ligated immediately above and below the transection were fully perfused with blood, indicating the existence of a blood supply to the cervix from both uterine and vaginal sources. In each case, examination of the uterus revealed the membranes to be intact and to contain a live fetus. The silicone rubber disks attached to the transected ends of the cervix remained in position, occluding the cervical lumen.

The internal structure of the cervices removed from the animals in which labor had been induced (ewes 1 through 4) was found to have altered in a way similar to that seen at term. The rings of the proximal (uterine) portions were effaced, with liquid and translucent mucus present. Structural alteration of the distal (vaginal) portions had not progressed as far although similar

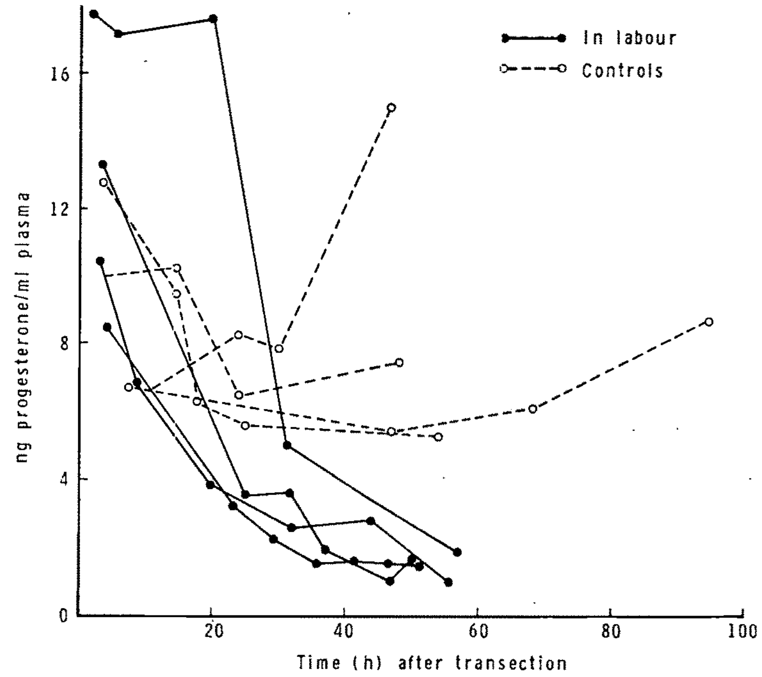

Fig. 2. Graph of the concentrations of progesterone in peripheral plasma collected from eight ewes following cervical transection after intrafetal injection of dexamethasone (ewes 1 through 4) or sterile normal saline solution (ewes 5 through 8).

changes had clearly taken place, with increased cervical dimensions, loss of rigidity, and liquid mucus observable. Fig. 4 shows a photograph of one cervix from this group. Cervices taken from control ewes were similar in appearance to the normal structure seen during late pregnancy and there were no visible differences between the uterine and vaginal portions.

Extensibility measurements. Within 30 minutes of the sacrifice, tissues were mounted in the apparatus used for determination of extensibility. Measurements were made on the central cervical ring removed from the cervix at postmortem examination. Results of these experiments are shown in Table I. Cervices taken from four ewes in which labor had been induced each showed an increase in extensibility compared to measurements made on control cervices. The results obtained from the control group did not differ from extensibilities measured on nontransected cervices taken from sheep of similar gestational age.[4] Extensibilities measured for the group in which labor had been induced were always at least twice as great as the highest figure obtained for the control group. Statistical analysis was not performed.

Comment

The purpose of this study was to determine the effect of labor on the cervix that has been surgically isolated from the uterus while retaining a connection with the vagina. No effect was seen in the absence of labor. In the ewes in which labor had been induced, cervical softening still occurred after transection, although the response of the distal (vaginal) portion appeared less marked than that of the portion that remained in contact with the uterus. During operation, particular care was taken to sever all visible vessels in the plexus lying around the cervix. It was clear at postmortem examination that both halves of the transected plexus still received a blood supply, an observation supported by the absence of necrosis in the two portions. The attenuation of the response might have been due to the absence of prostaglandins that could otherwise have reached the cervix by venous drainage from the uterus or to the removal of the influence of uterine contractions on the cervix. Cervical transection did not appear to attenuate the amplitude or frequency of the uterine contractions induced with dexamethasone.

The effect of surgical transection of the cervix has not been previously studied in the sheep, although Stys et al.[3] have measured the compliance of the sheep cervix during labor following ligation of the internal os uteri with a purse-string suture. This procedure may have occluded the vascular connections between the uterus and cervix but would not affect the transmission of the forces of uterine contractions to the cervix. Despite the insertion of the purse-string suture, cervical compliance increased considerably during both spontaneous and dexamethasone-induced labor.

Results similar to the present study have been re-

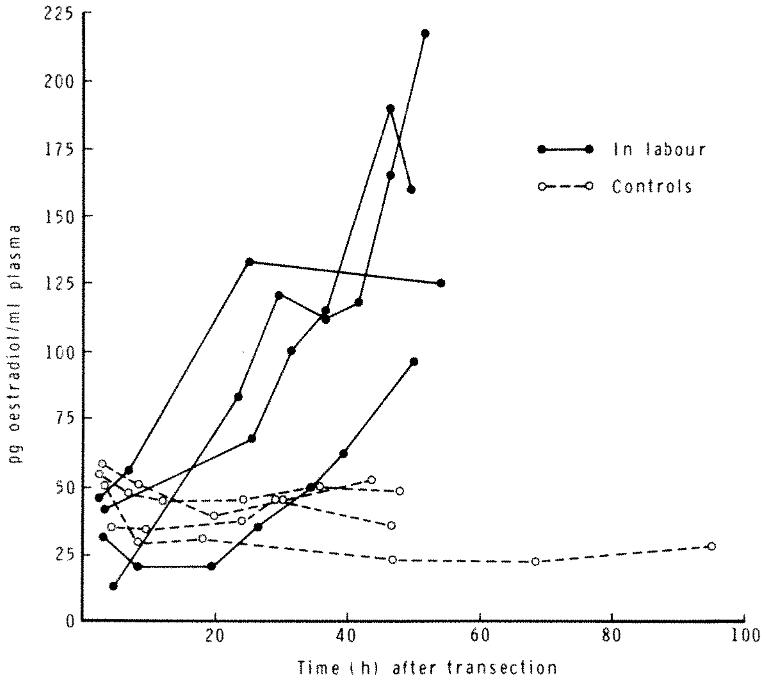

Fig. 3. Graph of the concentrations of 17β-estradiol in peripheral plasma collected from eight ewes following cervical transection after intrafetal injection of dexamethasone (ewes 1 through 4) or sterile normal saline solution (ewes 5 through 8).

Table I. Extensibilities of cervical strips taken from distal (vaginal) portions of transected cervices following labor induced with dexamethasone or from controls

Ewe No.	*Treatment*	*Cervical extensibility*
1	Dexamethasone	7.9
2	Dexamethasone	10.9
3	Dexamethasone	12.8
4	Dexamethasone	12.1
Mean		10.9
5	Saline solution	4.0
6	Saline solution	2.3
7	Saline solution	2.6
8	Saline solution	3.2
Mean		3.1

ported following transection of the cervix of the pregnant rat. Hollingsworth and Gallimore[12] measured the wet weight and extensibility of the cervix 8 days after transections performed on day 11 of pregnancy. Both of these measurements have been shown to increase during the latter half of pregnancy in the rat[13] and changes of a similar magnitude were still found to occur after transection. The authors concluded that cervical softening during pregnancy was not a consequence of an increase in uterine contractility.

The present study has extended the findings of Hollingsworth and Gallimore[12] to changes in the cervix during labor. It would appear that the cervix of the pregnant sheep can still undergo the structural remodeling that leads to softening of the tissue after its separation from the influence of uterine contractions. This finding adds to the evidence contradicting the hypothesis that cervical softening is the result only of stretching of the tissue by the force of the contractions of the uterus. The cervix of the sheep can produce prostaglandins in vitro. Production of prostaglandin E and 6-oxo-prostaglandin $F_{1\alpha}$ (the stable breakdown product of prostacyclin) is increased in tissues collected at delivery.[14] It is possible that in the present experi-

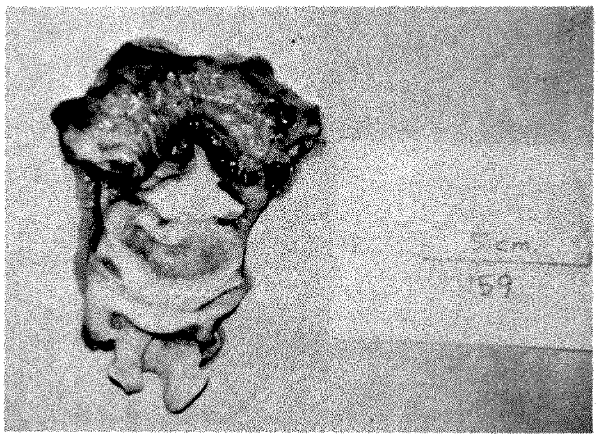

Fig. 4. Photograph shows a transected cervix shortly after sacrifice of ewe 1 during labor. The uterine portion of the cervix lies at the top of the picture.

mental circumstances, cervical prostaglandin synthase activity may have been enhanced in response to changes in the concentrations of steroid hormones in the cervical vascular supply by a mechanism similar to that suggested for the control of uterine prostaglandin production.[15] It is less likely that the observed response of the cervical tissue was the result of prostaglandins produced by the uterus passing to the cervix in the systemic circulation, since these compounds are efficiently metabolized by the lungs and other organs.[16] Release of relaxin into the circulation from the corpus luteum or from an extraovarian site of production might also participate in the process of cervical softening and may also remain unaffected by transection. In the intact animal, prostaglandins and mechanical factors from the uterus may combine with such factors to produce a fully dilated cervix, but in their absence it would appear that the initial softening phase of the process of dilatation can still proceed.

We thank Professor G. S. Dawes for provision of animal housing facilities.

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Human fetal breathing: Associated changes in heart rate and beat-to-beat variability

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A computerized quantification of fetal heart rate and beat-to-beat variability was performed during and between fetal breathing episodes. Sixteen healthy pregnant women at term participated in the study. The results indicate that fetal breathing is associated with a significant increase in beat-to-beat variability (from 4.48 ± 1.66 between breathing episodes to 5.80 ± 2.04 during breathing episodes, $p < 0.01$) and a small but significant decrease in fetal heart rate (from 142.7 ± 8.6 bpm between breathing episodes to 140.4 ± 8.6 bpm during breathing episodes, $p < 0.05$). (AM J OBSTET GYNECOL 1985;151:403-6.)

Key words: Fetal breathing, fetal heart rate, heart rate variability

The assessment of fetal heart rate variability and its significance to fetal well-being have received increased attention in recent years.[1] To date, there have been few quantitative investigations of specific mechanisms responsible for such variations.

It has long been recognized that during extrauterine breathing, variations in heart rate occur simultaneously with breathing (i.e., respiratory sinus arrhythmia).[2] This study was undertaken in order to quantify changes in fetal heart rate and beat-to-beat variability during and between fetal breathing episodes.

Material and methods

Informed consent was obtained from 16 healthy pregnant women at term in whom spontaneous rupture of the membranes had occurred. They were all normal in that there were no complications of pregnancy, they were nonsmokers and took no drugs (other than ferrous compounds). The study was performed at least 2 hours after spontaneous rupture of membranes (range, 2 to 7 hours) and before the onset of uterine contractions. All records were of 25 minutes duration and were made at midmorning, 2 hours after a standard breakfast.

Fetal activity was monitored by an observer using a real-time ultrasound scanner. The transducer was placed on the maternal abdomen and a view obtained of a transverse section of the upper fetal abdomen. Two switches were operated by the observer; one was actuated for every breathing movement, and the other was actuated throughout each fetal body movement. Hiccup-type movements and isolated limb movements were not recorded. The fetal electrocardiograms were obtained via a scalp electrode, amplified, and continuously recorded. All data input was simultaneously recorded on three channels of a four-channel FM tape recorder. On replay the information was fed into a microcomputer with the use of a sampling time of 0.1 msec. A detailed description of this system forms the subject of a separate publication.[3]

The fetus was defined as breathing when the respiratory rate was ≥ 10 breaths per minute. Absence of breathing for >6 seconds was defined as apnea.

Beat-to-beat variability was defined as follows:

$$\frac{[\mathrm{Tn} - \mathrm{T}(\mathrm{n} - 1)] \times 1000}{\mathrm{Tn} + \mathrm{T}(\mathrm{n} - 1)}$$

where Tn = the n^{th} interbeat time interval.[4]
A numerical analysis was performed on the data input, yielding the following items: (1) Fetal breathing—number of breathing episodes and percentage of time during which fetus was breathing. (2) Fetal heart rate—derived from beat-to-beat intervals ($F = 1/T$) during periods of fetal breathing and of no fetal activity. (3) Beat-to-beat variability—defined as the standard deviation of the equation given above and occurring during periods of fetal breathing and of no fetal activity. The Wilcoxon matched-pair signed-rank test was used for statistical analysis.

Results

Fetal breathing. The number of breathing episodes and percent of observation time of these episodes for each patient is presented in Table I. A total of 106 breathing episodes, lasting for 5961 seconds, were analyzed in the study.

Fetal heart rate. Mean values for fetal heart rate during and between fetal breathing episodes were 140.4 ±

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Table I. Fetal breathing

Case No.	Percent of observation time*	No. of breathing episodes
1	11.2	2
2	48.6	8
3	14.5	2
4	16.5	4
5	16.8	4
6	51.0	8
7	18.1	7
8	11.4	9
9	12.7	4
10	13.7	7
11	54.9	9
12	10.6	8
13	16.8	6
14	9.7	6
15	16.9	12
16	74.0	10

*Mean, 24.8; SEM, 6.2.

Table II. Changes in fetal heart rate associated with fetal breathing episodes

	Fetal heart rate (bpm)	
Case No.	During fetal breathing episodes*	During periods of no fetal activity†
1	124.8	137.9
2	158.1	164.2
3	137.1	140.1
4	140.1	139.5
5	140.8	142.6
6	142.2	145.5
7	130.8	140.2
8	128.8	128.6
9	142.0	141.0
10	136.0	136.8
11	141.9	142.0
12	136.5	134.9
13	158.9	162.0
14	143.7	144.1
15	144.6	144.5
16	140.3	140.6

*Mean, 140.4; SEM, 2.15.
†Mean, 142.7; SEM, 2.15.

8.6 and 142.7 ± 8.6, respectively (mean ± SD, bpm). This difference is significant ($p < 0.05$). A summary of the results appears in Table II.

Beat-to-beat variability. Mean values for beat-to-beat variability during and between fetal breathing episodes were 5.80 ± 2.04 and 4.48 ± 1.66, respectively (mean ± SD). This difference is significant ($p < 0.01$). A summary of the results appears in Table III. Fig. 1 is a typical example of fetal breathing movements along with the corresponding fetal heart rate and beat-to-beat variability.

Table III. Changes in beat-to-beat variability associated with fetal breathing episodes

	Beat-to-beat variability	
Case No.	During fetal breathing episodes*	During periods of no fetal activity†
1	9.35	7.10
2	5.11	3.20
3	3.40	2.68
4	6.70	6.06
5	9.98	6.50
6	5.82	3.31
7	5.78	6.30
8	5.78	4.41
9	3.85	3.45
10	4.45	4.11
11	3.75	2.55
12	9.22	7.12
13	5.11	3.17
14	3.85	2.72
15	4.06	3.04
16	6.71	6.08

*Mean, 5.80; SEM, 0.51.
†Mean, 4.48; SEM, 0.41.

Fetal outcome. The outcome of each of the pregnancies was entirely normal. No fetus developed signs of distress during the intrapartum period. All delivered vaginally. There were no Apgar scores <8 at 1 and 5 minutes. Neonatal examination confirmed gestational age. The average fetal weight was 3540 gm (range, 3150 to 3790 gm).

Comment

A computerized numerical analysis of fetal heart rate and beat-to-beat variability during and between fetal breathing episodes was carried out in this study. Our results indicate that beat-to-beat variability is significantly increased during fetal breathing. As seen, beat-to-beat variability rose from 4.48 ± 1.66 between breathing episodes to 5.80 ± 2.04 during breathing episodes (mean ± SD, $p < 0.01$). Fetal heart rate dropped to 140.4 ± 8.6 bpm from 142.7 ± 8.6 bpm during and between breathing episodes, respectively ($p < 0.05$).

The association between fetal breathing movements and a change in the pattern of fetal heart rate was noted but not quantified by Timor-Tritsch et al.[5] Wheeler et al.[6] found an increase in both short- and long-term beat-to-beat variability with fetal breathing. In contrast, Botts et al.[7] found no significant association between fetal breathing movements and either form of beat-to-beat variability. Dawes et al.[8] examined fetal heart rate via abdominal electrocardiogram in the presence of fetal breathing only during periods when there were neither gross accelerations nor decelerations >3.5 bpm. They looked at the absolute beat-to-beat intervals and

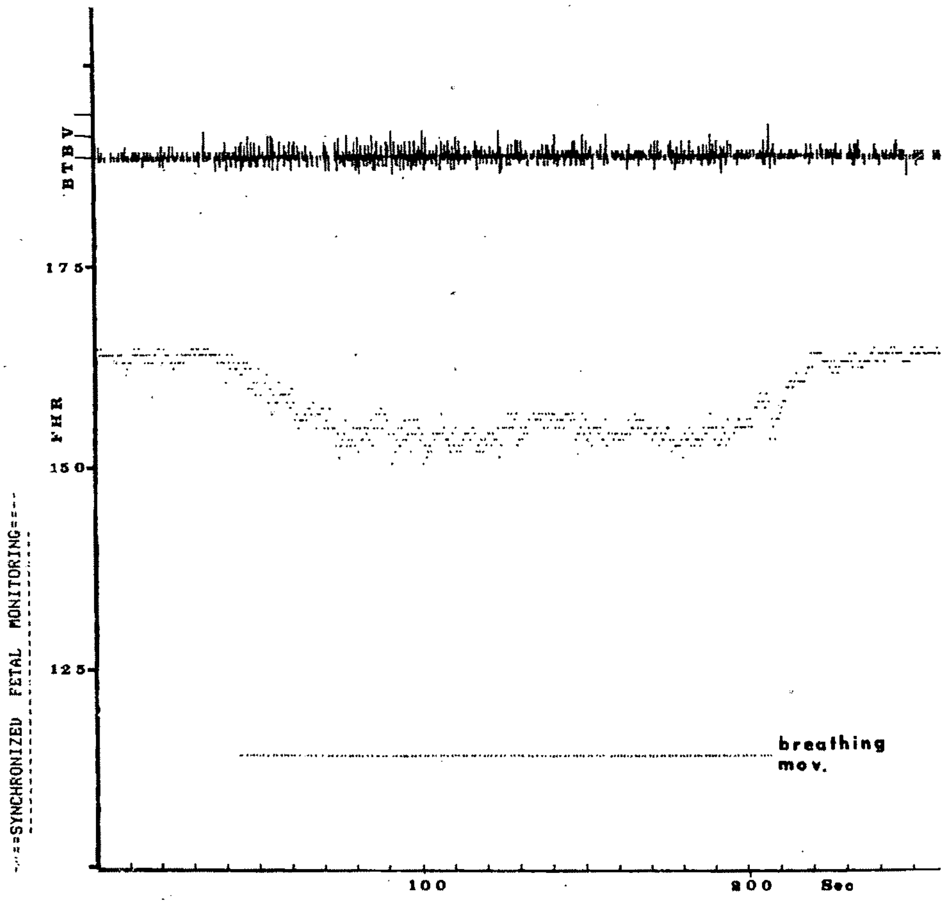

Fig. 1. Fetal breathing movements along with the corresponding fetal heart rate *(FHR)* and beat-to-beat variability *(BTBV)*.

found a mean increase of 1 msec in their short-term beat-to-beat variability, whereas the mean fetal heart rate fell by 2.8 bpm. In our study, we examined beat-to-beat variability during fetal breathing, without imposing any limits on fetal heart rate fluctuations. Our findings support previous results, which showed that fetal breathing is associated with a significant increase in beat-to-beat variability and a small, but significant decrease in fetal heart rate.

In contrast to previous investigators who looked at the relationship between fetal heart rate, beat-to-beat variability, and fetal activity using fetal abdominal electrocardiogram or Doppler ultrasound,[5, 6, 8] we used the direct fetal electrocardiogram as the source for calculating beat-to-beat variability. Since the accuracy of these measurements depends on the accuracy of the trigger signal, we chose the direct electrocardiogram from the fetal scalp. Thus we could calculate fetal heart rate and beat-to-beat variability directly and more accurately.

All of our subjects were examined after spontaneous rupture of membranes and before the onset of uterine contractions. This is clearly a unique situation. The reason for choosing this experimental setup is that it is as free of artifacts as possible. With the use of the direct fetal electrocardiogram as the trigger source and an error detection system described elsewhere,[3] our total rejection time resulting from artifacts was <0.2% per recording. Because the signals were of such good quality, we did not have to perform any filtering, averaging, or interpolation, as was done by others.[8]

The clinical significance of the information presented in this paper has yet to be determined. However, it seems reasonable that a combined assessment of fetal breathing, and its associated changes in fetal heart rate and beat-to-beat variability may prove useful in evaluation of fetal well-being. This assumption is based on the following facts: (1) Fetal biophysical activities are initiated and regulated by complex integrated central nervous system mechanisms.[9] (2) The mechanisms involved in the association between breathing and changes in heart rate, i.e., respiratory sinus arrhythmia, are fairly well understood.[10, 11] (3) As far as our ability to stress the system is concerned, it is relatively easy to enhance fetal breathing movements (e.g., by maternal glucose administration).[12]

Studies are presently under way to investigate the effects of asphyxia and drug administration on "respiratory sinus arrhythmia" in the human fetus.

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Disposition of meperidine and normeperidine following multiple doses during labor

I. Mother

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Normeperidine, the active meperidine metabolite, has been implicated in adverse neonatal effects that may occur following administration of meperidine to parturients. However, recent studies have suggested that normeperidine levels are not high enough to have adverse effects following single low doses of meperidine. It is not clear what occurs following multiple injections. Therefore the purpose of this study was to quantitate plasma levels of meperidine and normeperidine in the mother following multiple doses of meperidine over long time periods and to determine the half-life of normeperidine. Twelve mothers who received multiple intravenous doses of meperidine were studied. The results show that both meperidine and normeperidine accumulate in maternal plasma following multiple injections and that the half-life of normeperidine averages 20.6 hours. The data suggest that maximum exposure of the fetus to both meperidine and normeperidine would result from multiple doses to the mother because of a continued diffusion gradient from mother to fetus. (AM J OBSTET GYNECOL 1985;151:406-9.)

Key words: Mother, pregnancy, drugs, meperidine

Normeperidine, the pharmacologically active meperidine metabolite, has been implicated as the cause of respiratory depression in infants born 2 to 3 hours following meperidine administration to the mother.[1] However, recent studies have suggested that following single doses and short drug-to-delivery intervals, depression in the newborn infant appears to be related more to the total amount of meperidine transferred to

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the fetus before delivery than to the presence of normeperidine.[2-7]

Normeperidine may have a clinical effect, however, if meperidine is given to the mother as repeated injections with long drug-to-delivery intervals.[6] The fact that normeperidine is excreted more slowly than meperidine supports this hypothesis.[8, 9] Although the half-life in parturients is not well documented, normeperidine would be expected to accumulate in maternal as well as fetal plasma following multiple doses.[8, 9] Thus an infant born to a mother who has received several injections of meperidine over long periods of time may have considerable normeperidine levels at birth.

Therefore the purpose of this study was to document the disposition of meperidine and normeperidine in maternal plasma following multiple doses of meperidine and to determine the half-life of normeperidine in these patients.

Material and methods

Patients. Twelve pregnant women were studied. Meperidine was administered intravenously as needed for pain relief. Patients with liver or renal disease or histories of substance abuse were eliminated from the study. Others with mild pregnancy complications were included as long as meperidine administration was appropriate. This study was approved by the Human Investigation Committee of Cleveland Metropolitan General Hospital, and appropriate informed consent was obtained from each patient prior to inclusion in the study.

Sample collection and data analysis. Intrapartum maternal blood samples were collected at various intervals following meperidine injection as previously described.[10, 11] Postpartum blood samples were collected from four of the mothers every 24 hours for 72 hours.

Meperidine was extracted from plasma with the use of the technique of Mather and Tucker[12] and quantitated by gas chromatography–mass spectrometry.[7]

Semilogarithmic plots showing the disappearance of meperidine and normeperidine in maternal plasma were obtained by plotting concentrations of drugs in plasma against the time since injection of meperidine. Half-lives were estimated from the disappearance of the compounds in plasma. Normeperidine/meperidine ratios were calculated by dividing the amount of normeperidine in a given sample by the amount of meperidine in the same sample. Statistics and calculations were done by means of the statistical and linear or exponential curve–fitting programs for a Hewlett Packard 41C programmable calculator.

Results

Patient population. The clinical characteristics and amount of meperidine administered to the mothers are shown in Table I. One cesarean section was performed because of fetal distress and the second for failure to progress following premature rupture of membranes. The neonatal outcomes of these pregnancies are presented in a companion paper.[13]

Table I. Characteristics of the maternal study population

Characteristic	*Number*
No. of mothers	12
Antenatal characteristics	
Age (yr)*	22.6 ± 4.5 (17-32)
Nulliparous	9
Multiparous	3
Intrapartum analgesia	
Meperidine (No. of doses)	2.58 ± 0.79 (2-4)
Total dose (mg)	79.04 ± 25.9 (50-125)
Drug-to-delivery interval: first dose (min)	303 ± 190 (93-702)
Drug-to-delivery interval: final dose (min)	150 ± 110 (51-401)
Delivery method (n)	
Vaginal	10
Primary cesarean section	2
Complications (n)	
Preeclampsia	3
>200 lb	1
Sickle cell disease	1
Smoking	4
Premature rupture of membranes	1

*Mean ± SD (range).

Intrapartum plasma drug levels. A disappearance curve from a typical patient resulting from three successive injections of 25 mg of meperidine at hourly intervals and the corresponding appearance curves of normeperidine are shown in Fig. 1. These curves show that both meperidine and normeperidine plasma levels steadily increase with time. Plasma levels of meperidine an hour after the second or third dose were typically two to three times higher than those at 1 hour after the first dose. Plasma levels of normeperidine increased at variable rates in individual patients. No differences were apparent in patients with medical complications.

The mean maternal plasma meperidine and normeperidine levels at delivery and the normeperidine/meperidine ratio are shown in Table II. A delivery sample was not obtained from one patient. No significant correlation was observed between normeperidine levels and the drug-to-delivery interval. However, the proportion of normeperidine to meperidine increased significantly ($r = 0.77$, $p < 0.01$, $Y = 0.03 + 0.000753X$) with time; the high observed was 58%. Fig. 2 illustrates this relationship.

Postpartum plasma drug levels. Samples were collected from four mothers post partum and the mean half-life of normeperidine was estimated to be 20.55 ± 3.61 hours (range, 17.3 to 24.8 hours). At 24 hours

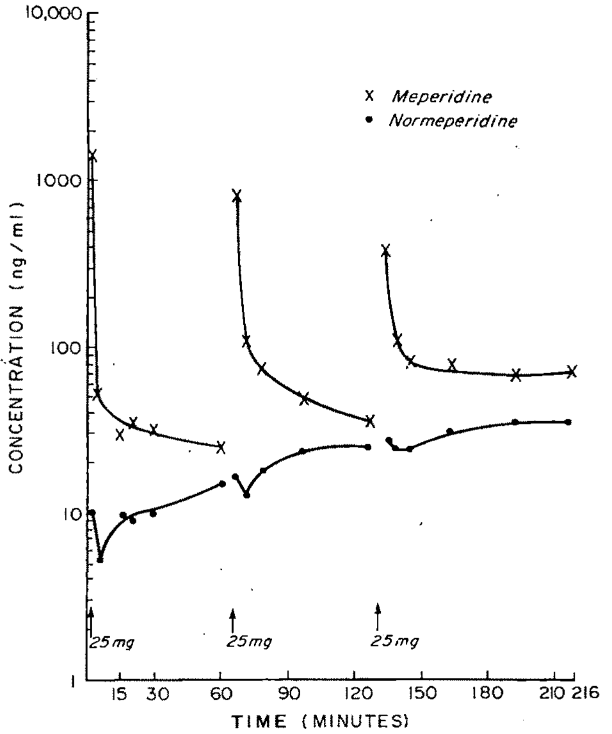

Fig. 1. Plasma concentrations of meperidine and normeperidine following multiple doses of meperidine during labor.

Table II. Maternal meperidine and normeperidine levels (ng/ml) and the normeperidine/meperidine ratio at delivery

	Mean ± SD	*Range*
Meperidine	129.2 ± 57.5	65.6-268.2
Normeperidine	33.6 ± 19.9	10.5-69.1
Normeperidine/meperidine*	0.26 ± 0.17	0.09-0.58

*Corresponds to an average injection-to-delivery interval of 275 min from the first dose.

post partum, plasma normeperidine levels were similar to those found at delivery, whereas meperidine levels were very low. For this reason, not enough data points were available to calculate the postpartum half-life of meperidine in these patients. However, in two of the mothers, meperidine was detectable for at least 48 hours.

Comment

Intrapartum plasma drug levels. Both meperidine and normeperidine levels in maternal plasma increase with repeated injections. Although this is not surprising, there may be implications for the fetus that may not have been considered. When a single dose of meperidine is given, uptake of meperidine into fetal tissues occurs for 2 to 3 hours, at which point the diffusion gradient reverses and meperidine begins to be cleared from fetal tissues.[2,5,6] When multiple doses of meperidine are given, uptake into fetal tissues occurs as before, but the concentration gradient does not have time to reverse before the next injection. Uptake of meperidine into fetal tissues would continue for at least 2 to 3 hours after the last dose.

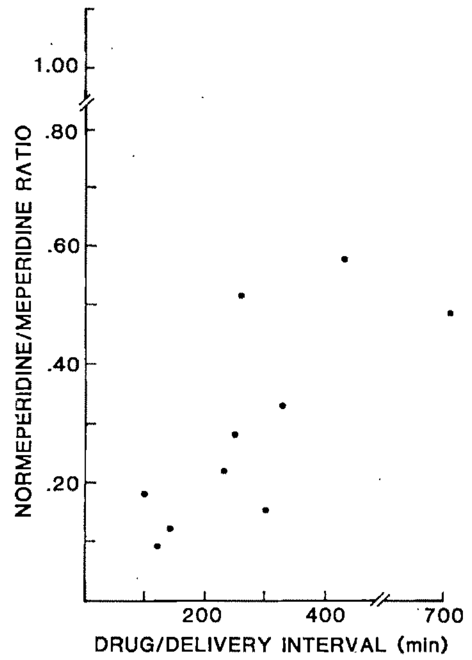

Fig. 2. The relationship between the normeperidine/meperidine ratio and the drug-to-delivery interval.

With respect to normeperidine, its level also increases in maternal plasma, and the proportion of normeperidine to meperidine increases with time. Therefore fetal tissue uptake of normeperidine by diffusion will continue, and fetal levels will be augmented by what is produced by fetal metabolism. Normeperidine should accumulate in fetal tissues for many hours following the last dose of meperidine. At the highest levels, normeperidine may be clinically relevant, since it could add to the effects of the meperidine already present.[6]

Postpartum plasma drug levels. The half-life of meperidine has consistently been reported to be approximately 3 to 7 hours.[4,5,10,14] However, several of the mothers in this study had detectable meperidine levels for as long as 48 hours. These results are in agreement with a recent study by Morrison et al.,[15] who also reported the presence of meperidine in maternal plasma for as long as 48 hours. The results of these two studies suggest that the postpartum half-life of meperidine in maternal plasma may have been underestimated by earlier investigators.

To our knowledge, the absolute half-life of normeperidine in the puerperium has not previously been published although several studies have suggested that it is "prolonged."[4,11,15] Finding a mean half-life of >20 hours for these patients suggests that normeperidine would still be increasing in maternal plasma for hours after delivery of the infant.

In summary, the results of this study suggest that with multiple doses, the drug and its metabolite would continuously accumulate in the fetus because of the continued diffusion gradient from mother to fetus. This would result in maximum accumulation of both compounds in fetal tissues at birth.

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Disposition of meperidine and normeperidine following multiple doses during labor

II. Fetus and neonate

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It has been suggested that continued diffusion gradients from mother to fetus would exist and that both meperidine and normeperidine would accumulate in the fetus following multiple doses of meperidine to the mother during labor. However, no pharmacokinetic data are available. Therefore, the purpose of this study was to document the disposition of meperidine and normeperidine in the fetus and neonate following multiple doses of meperidine to the mother over long time periods. Twelve infants were studied. The results show surprisingly high concentrations of both meperidine and normeperidine in fetal blood at delivery. In addition, the amount of normeperidine increased with time in umbilical cord blood, the ratio of normeperidine to meperidine increased with time, and the umbilical artery-to-vein ratio of meperidine (but not normeperidine) was greater than one following long drug-to-delivery intervals. The data also suggest that with long drug-to-delivery intervals the levels of normeperidine may become clinically important and that the elimination of both compounds by the neonate is prolonged. The study suggests that multiple doses to the mother over long time periods result in maximum accumulation of both meperidine and normeperidine in fetal tissues. (AM J OBSTET GYNECOL 1985;151:410-5.)

Key words: Fetus, drugs, meperidine

Infants born 2 to 3 hours following meperidine administration to the mother have repeatedly been shown to have the highest incidence of respiratory depression.[1-3] Since no relationship could be demonstrated between cord blood meperidine levels and this time interval,[1, 4] it was hypothesized that normeperidine, the pharmacologically active metabolite of meperidine,[5] was responsible. However, normeperidine levels are too low to explain any respiratory depression in the neonate following single low doses of meperidine.[6-13] Furthermore, fetal tissue uptake of meperidine, but not normeperidine, reaches a maximum approximately 2 to 3 hours following its administration to the mother[6, 9, 10]; in contrast, normeperidine levels are highest with longer time intervals.[10, 11] Thus, following single doses and short drug-to-delivery intervals, depression in the newborn appears to be related more to the total amount of meperidine transferred to the fetus prior to delivery than to the presence of normeperidine.

However, normeperidine may be clinically relevant if meperidine is given to the mother as multiple doses with long drug-to-delivery intervals.[10] This is supported by the accumulation of normeperidine in maternal plasma, particularly following multiple doses.[14] Thus an infant born to a mother who has received several injections of meperidine over long periods of time may have considerable normeperidine levels at birth. Furthermore, neonatal clearance of normeperidine is much slower than in the adult,[8, 9, 15-17] and normeperidine is not completely antagonized by naloxone.[18]

Therefore the purpose of this study was to document the disposition of meperidine and normeperidine in fetal and neonatal plasma and urine following multiple doses of meperidine during labor.

Material and methods

Patients. Twelve neonates whose mothers received multiple low doses of meperidine during labor were studied. Meperidine was administered intravenously to the mothers as needed for pain relief. The clinical characteristics of the mothers, the timing and amount of analgesia, and the informed consent procedures have been reported previously.[14]

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Sample collection and data analysis. Cord blood samples were collected from 11 of the infants as described.[6, 15] Blood samples were collected from five of the neonates every 24 hours for 72 hours. Neonatal urine samples were also collected as described.[6] The goal for each neonate was to obtain six consecutive 12-hour samples. However, neonatal collections were stopped because of skin irritation associated with the collection bags in six of the infants. Data from urine samples are expressed as micrograms of drug excreted. Meperidine was extracted from plasma and urine as previously described.[14]

Semilogarithmic plots showing the disappearance of meperidine and normeperidine in neonatal plasma were obtained by plotting concentrations of drugs in plasma against time since birth. Plasma half-lives were estimated. Fetal/maternal ratios were calculated by dividing the amount of meperidine or normeperidine in the cord vein at birth by the amount in the maternal vein at birth. Ratios of cord artery to cord vein were calculated by dividing the level of drug in the cord artery by the level found in the cord vein. Normeperidine/meperidine ratios were calculated by dividing the amount of normeperidine in a given sample by the amount of meperidine in the same sample.

Results

Patient population. The neonatal clinical characteristics are summarized in Table I. One neonate had Apgar scores of 1 at 1 minute and 7 at 5 minutes. This neonate was delivered by emergency cesarean section under general anesthesia following prolonged fetal heart rate decelerations. There was thick meconium in the amniotic fluid, and a fetal scalp blood pH value prior to birth was 7.18. The mother had received three doses of meperidine (125 mg total) with a drug-to-delivery interval of 426 minutes from the first dose and 207 minutes from the last. At delivery the levels of meperidine and normeperidine in the cord vein were 110 and 70.68 ng/ml, respectively. The fetal vein/maternal vein ratios were 0.94 and 1.05, respectively. This infant had the highest level of normeperidine found in the study. The infant responded to a narcotic antagonist and was discharged after an uneventful stay in the nursery.

A second infant had 1- and 5-minute Apgar scores of 2. This fetus demonstrated late decelerations in the second stage of labor. After a measurement of fetal scalp blood pH of 7.32 the patient continued to labor. Subsequently there was a fetal heart rate deceleration to 60 bpm and a midforceps delivery. The neonate had the umbilical cord wrapped tightly around her neck three times, was asphyxiated, and required intubation. Generalized seizures began approximately 20 minutes after delivery. The mother had received two intravenous doses of meperidine (50 mg total) with the first 25 mg dose at 223 minutes and the second at 208 minutes prior to delivery. Cord vein levels at delivery were 72.96 and 14.86 ng/ml for meperidine and normeperidine, respectively. The fetal vein/maternal vein ratios were 1.12 and 1.04, respectively. Narcotics were not thought to be related to the infant's depression. At discharge the infant had an abnormal neurological examination and was receiving phenobarbital.

Table I. Characteristics of the neonatal study population

Characteristic	*Number*
No. of infants	12
Gestational age (wk)	39.9 ± 1.2 (37-41)
Birth weight (gm)*	3306 ± 501 (2320-4090)
Apgar score <7 (n)	
1 min	2
5 min	1
Complications (n)	
<37 weeks' gestation	1
Cord entanglement	2
Asphyxia	2
Narcotic antagonist	1
Large for dates	1
Small for dates	1
Abnormal at discharge (seizures)	1

*Mean ± SD (range).

The mean, standard deviation, and range of the meperidine and normeperidine levels in umbilical vein and artery are shown in Table II. A significant relationship was observed between the first injection-to-delivery interval and cord vein normeperidine levels ($r = 0.72$, $p < 0.5$; regression equation, $Y = -2.31 + 0.13X$).

Table II also shows the fetal vein/maternal vein ratios for both meperidine and normeperidine. There were three infants with meperidine fetal vein/maternal vein ratios >1, and two of these also had normeperidine fetal vein/maternal vein ratios >1. All three infants had clinical signs of acidosis and/or the lowest 1-minute Apgar scores in the study. No significant relationships were found between the fetal vein/maternal vein ratios of either compound and the interval between the first administration of the drug and delivery.

The mean and range of the umbilical artery/umbilical vein ratio of meperidine and normeperidine are shown in Table II. The mean drug-to-delivery intervals for the mothers of these infants was 242 ± 77 minutes. Four of five infants had more meperidine in umbilical artery plasma than in umbilical vein; the one infant who did not have more had the only short drug-to-delivery interval (137 minutes). Only one of the five

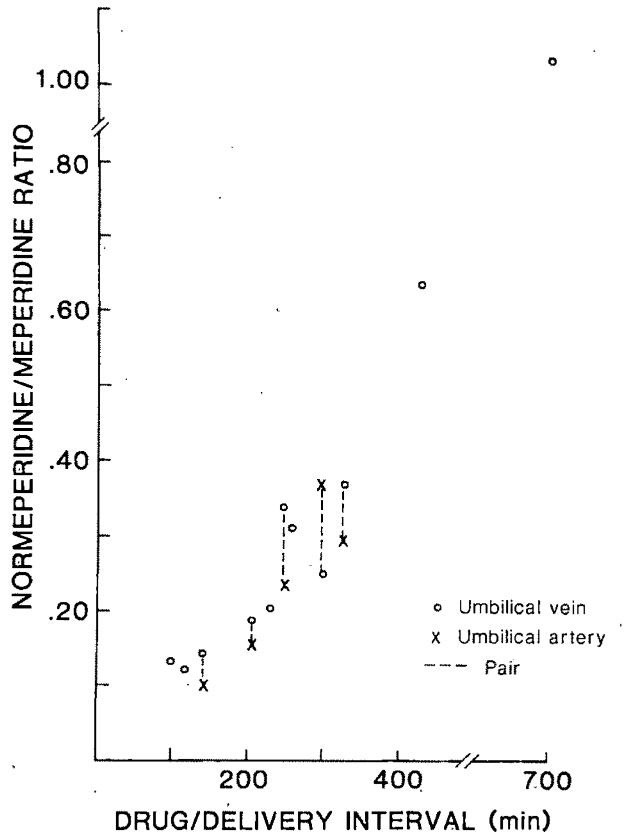

Fig. 1. The relationship between the normeperidine/meperidine ratio and the drug-to-delivery interval.

infants had more normeperidine in umbilical artery than in umbilical vein; this infant's drug-to-delivery interval was 298 min.

The proportion of normeperidine to meperidine in fetal blood increased significantly with time (Fig. 1) ($r = 0.96$, $p < 0.01$ [$Y = -0.21 + 0.00214X$] and $r = 0.91$, $p < 0.01$ [$Y = -0.07 + 0.00126X$] for umbilical vein and artery, respectively). The mean (and range) normeperidine/meperidine ratios in umbilical vein and artery respectively at delivery were 0.37 ± 0.38 (0.12 to 1.43) and 0.23 ± 0.11 (0.10 to 0.37). One infant was found with more normeperidine than meperidine in the umbilical vein at delivery. This infant's normeperidine/meperidine ratio was 1.43 following a drug-to-delivery interval of 702 minutes from the first dose and 216 minutes from the second of four 25 mg doses.

Neonatal plasma. Representative neonatal plasma profiles of meperidine and normeperidine concentrations for four of the infants are shown in Fig. 2, *A* to *D*. A fifth infant had a profile similar to that in Fig. 2, *C*. In all of the infants the meperidine level decreased rapidly and the level of normeperidine was higher than meperidine by the end of the second 24 hours of life. In two infants a definite increase in normeperidine level was seen in the first or second day (Fig. 2, *A* and *B*); in one infant the normeperidine level remained almost constant for over 72 hours (Fig. 2, *D*).

The half-lives of meperidine and normeperidine are shown in Table III. In two infants the normeperidine half-life could not be calculated because of the nonlinearity of the disappearance (see Fig. 2, *A* and *D*).

Neonatal urine. Three or more urine samples were collected from six infants during the first 72 hours of life. Complete collections from three of the infants suggested that 0.24% of the maternal dose is excreted by the infant in the form of meperidine and 0.11% in the form of normeperidine. When both compounds are combined, 0.36% of the maternal dose is excreted.

Fig. 3 illustrates the excretion of meperidine and normeperidine. Most of the meperidine is excreted before the first 24 hours, but both meperidine and normeperidine are still being excreted when the infants are discharged. Neither is excreted in a linear fashion. Meperidine excretion increases between 12 and 24 hours, whereas normeperidine increases steadily for the first 36 hours before decreasing.

Comment

Study population. The outcomes of two of the pregnancies are relevant to the study. Of particular interest is the infant with Apgar scores of 1 and 7 at birth who responded to the narcotic antagonist. The level of meperidine in this infant's cord vein was not exceptionally high compared to that of the rest of the study population, but the level of normeperidine was the highest noted. Furthermore, the fetal vein/maternal vein ratios were also among the highest noted. Although no conclusions can be drawn from the case of one infant with a high normeperidine level, it suggests that with long drug-to-delivery intervals a high level of normeperidine may result, which could certainly add to the effect of meperidine already present.

Also of interest is the severely asphyxiated infant whose asphyxia was not thought to be due to narcotic depression. In this case the fetal vein/maternal vein ratios were again very high even though the absolute levels of meperidine and normeperidine were low. This suggests that ion trapping of weakly basic drugs in the more acidotic fetal tissues may occur.[19, 20] It is well known that elevated concentrations of local anesthetic agents occur as a result of ion trapping in an acidotic environment,[19] and meperidine and normeperidine would be expected to behave similarly. These two cases suggest that more studies are warranted to consider the relationships between meperidine and normeperidine levels and neonatal outcome.

Umbilical cord plasma. Surprisingly high levels of both meperidine and normeperidine were found in

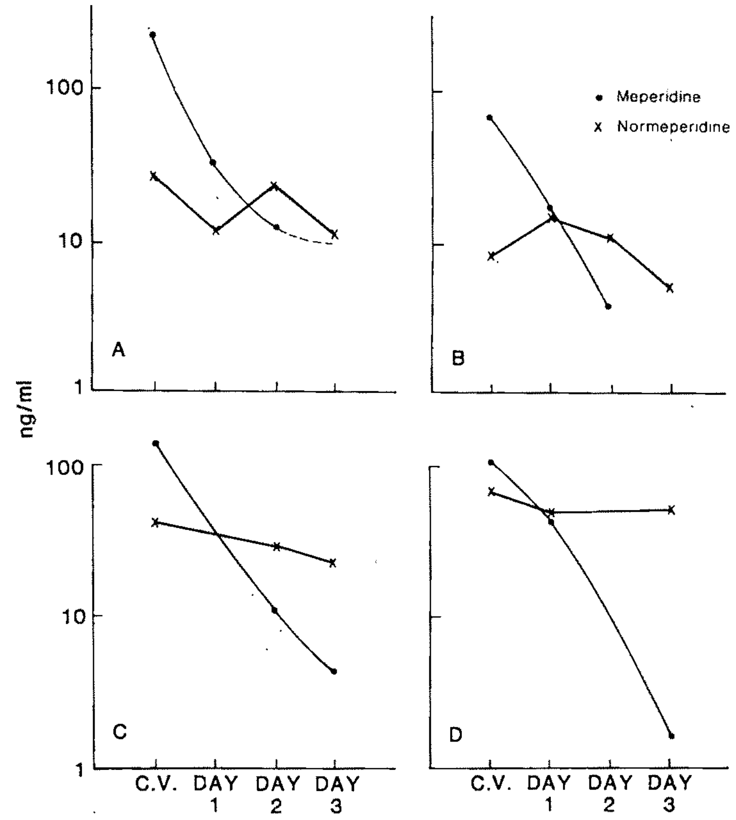

Fig. 2. Neonatal plasma concentrations of meperidine and normeperidine.

Table II. Umbilical vein and artery drug levels at delivery

	No.	*Meperidine**	*Normeperidine**
Umbilical vein	11	97.81 ± 53.8 (23-225.2)	27.91 ± 17.62 (8.1-70.7)
Umbilical artery	5	94.35 ± 32.94 (74-153)	23.59 ± 19.16 (8.1-56.7)
Umbilical artery/umbilical vein ratio	5	1.08 ± 0.15 (0.84-1.22)	0.99 ± 0.45 (0.59-1.76)
Fetal vein/maternal vein ratio†	11	0.73 ± 0.28 (0.31-1.16)	0.84 ± 0.15 (0.77-1.05)

*Mean ± SD in ng/ml (range).

†Mean of ratios, not ratio of means.

some of the fetal cord plasma samples at delivery. Levels as high as 225 ng/ml of meperidine and 71 ng/ml of normeperidine were found in umbilical vein at delivery following administration of only ≤125 mg to the mother as multiple low doses over long time periods. This level of meperidine is as high as that seen in maternal plasma shortly after an intravenous injection of 25 mg,[14] and the level of normeperidine is higher than that typically found in maternal plasma.[14] Furthermore, as reported previously, the levels of normeperidine increase with time[10, 11] as does the proportion of normeperidine to meperidine. This was aptly illustrated by the infant with 702-minute drug-to-delivery interval and the normeperidine/meperidine ratio of 1.43. These findings support the suggestion that accumulation of both compounds in fetal tissues will occur for hours following the last dose of meperidine to the mother. Furthermore, this study suggests that the levels of normeperidine cannot always be considered negligible as was once suggested.[21, 22]

The fetal vein/maternal vein ratios of meperidine and normeperidine are similar to what has been reported.[6, 8, 13, 15, 16] With long drug-to-delivery intervals, the fetal vein/maternal vein ratio for the metabolite was found to be higher than that of the parent compound, which has been reported following intravenous per-

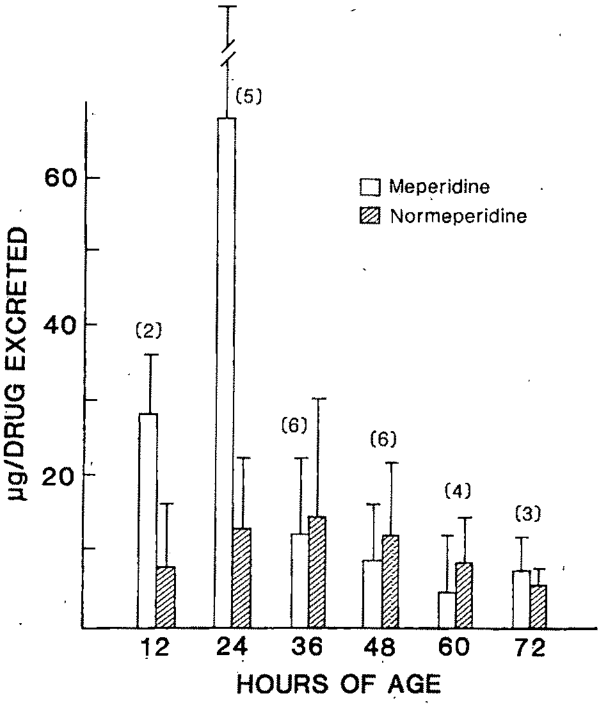

Fig. 3. Neonatal urinary excretion of meperidine and normeperidine.

fusion of meperidine.[8] This may be expected, since the metabolite diffusing across the placenta is added to what the fetus is producing.

The umbilical artery/umbilical vein ratios also support continued accumulation of both compounds with multiple doses. A ratio of 1 would mean that the fetal tissues and blood had reached equilibrium and is thought to occur about 2 hours following a single dose.[10] In all of the infants with drug-to-delivery intervals >3 hours, the meperidine ratio was >1. This suggests that considerable accumulation had occurred and that the diffusion gradient was now from fetal tissues back into fetal blood. (This was observed with an average drug-to-delivery interval from the first dose of 4 hours and from the last dose of 2 hours.) However, the normeperidine umbilical artery/umbilical vein ratio was >1 in only one infant, which suggests that normeperidine was still increasing and reaching equilibrium with fetal tissues. Higher levels of normeperidine in cord artery than in cord vein have been reported previously and also demonstrate formation of the metabolite by the fetus.[6, 8]

Neonatal plasma. In neonatal plasma the half-lives of both compounds are prolonged. The plasma meperidine half-lives ranging from 11 to 17 hours are in good agreement with previous reports ranging from 6.5 to 15 hours[8] or 12 to 39 hours.[16] The half-lives of normeperidine (29-85 hours) were more prolonged than that found in one other study.[8] Furthermore, these authors have also noted the increase in normeperidine levels 24 to 48 hours after birth.[8] These prolonged levels of normeperidine may have clinical significance[9, 10] and may help to explain some of the adverse effects noted days after delivery.[23]

Table III. Neonatal half-lives of meperidine and normeperidine

Patient No.	*Half-life in hours*	
	Meperidine	*Normeperidine*
1	11.36	—
2	11.55	28.88
3	17.33	74.52
4	14.44	84.51
5	11.55	—
Mean ± SD	13.24 ± 2.6	62.63 ± 29.7

Neonatal urine. In neonatal urine the prolonged excretion of meperidine and the prolonged and nonlinear excretion of normeperidine are consistent with the plasma disappearance of these compounds. Furthermore, the results of this study are similar to what has been reported previously following shorter drug-to-delivery intervals.[6, 15, 24] However, this study also adds the datum that 0.36% of the maternal dose is excreted by the neonate in the forms of meperidine and normeperidine during the first 72 hours of life.

In summary, the purpose of this study was to provide information regarding fetal and neonatal meperidine and normeperidine levels following multiple doses of meperidine to the mother over long periods of time. The results of this study show that with multiple doses the drug accumulates in fetal tissues because of the continued diffusion gradient from maternal to fetal tissues.[14] Furthermore, ion trapping can occur if the fetus is acidotic. Finally, with long drug to delivery intervals, the levels of normeperidine may become clinically significant because they can account for 60% or more of the total drug present.

The clinical protocol for the administration of meperidine during labor has changed as more has become known about the pharmacology of the drug in the intrapartum period.[25-27] The results of this and other recent studies[6, 10, 14] suggest that single doses are better than multiple doses for the fetus, since multiple dose regimens could lead to maximum accumulation of both meperidine and normeperidine in fetal tissues. Pharmacologically, the best time to be born following maternal analgesia with meperidine would be within 1 hour or >4 hours after a single, low intravenous dose.[6, 10, 11]

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CORRESPONDENCE

The frustrations of chronic pelvic pain

To the Editors:

I would like to address a number of issues raised by Dr. Slocumb in his paper on chronic pelvic pain (Slocumb JC. Neurological factors in chronic pelvic pain: trigger points and the abdominal pelvic pain syndrome. AM J OBSTET GYNECOL 1984;149:536). The subject is most important because there is a large number of women whose extensive complaints cannot be diagnosed precisely and whose treatment remains unsatisfactory.

I agree with Dr. Slocumb that conditions such as functional ovarian cysts, pelvic adhesions and congestion, the "universal joint syndrome" are, as causes of chronic pelvic pain, often merely figments of our frustration. I believe that the complaints may be psychosomatic, but this hypothesis is difficult to prove.[1] Certainly our patients generally detest the suggestion that their suffering is due to anything other than physical disease. Apart from reassurance and support, mainstream gynecology has little to offer in the care of these patients.

If Dr. Slocumb's success can be duplicated, patients and gynecologists will be indebted to him. However, success cannot be evaluated from a mixture of telephone calls, letters, and office visits. A great many treatments of this refractory condition have not stood the test of time. There may be a very substantial placebo effect from the care and treatments provided by Dr. Slocumb. Above all, Dr. Slocumb used trigger-point injections as well as supportive therapy and medications for insomnia, depression, and/or anxiety in his management. Which of these helped the most? Outcome should be asessed from carefully designed protocols and instruments, not from a mixture of treatments applied in undescribed proportions.

Questions should be asked about the pathologic conditions identified in the dorsal horns. A physical disease is postulated in an effort to explain the pain, but emotional factors are remarked upon, and thus psychosomatics are again invoked. Is there solid evidence of dorsal horn malfunction apart from subjective phenomena such as pain and hypersensitivity? Is it possible that higher centers produce whatever disease is identified in the dorsal horns? Has Dr. Slocumb given his patients another face-saving but scientifically suspect diagnosis?

A number of gynecologists have noted that chronic pelvic pain patients frequently present with multiple nongynecological symptoms such as headaches, lethargy, irritability, and depressive symptoms.[1-4] While these observations have been uncontrolled, more recent controlled studies have shown psychopathologic conditions in patients with chronic pelvic pain but no demonstrable disease.[5,6]

Much work remains to be done. Meanwhile, I hope that gynecologists will continue to perform laparoscopies instead of producing diagnoses unsupported by inspection of the internal genitalia. Dr. Slocumb's success with chronic pelvic pain patients appears to be impressive. If his methods can be duplicated, we should proceed to investigate the mechanisms involved.

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Reply to Strausz

To the Editors:

I would like to thank Dr. Strausz for his questions and comments concerning the paper on abdominal-pelvic pain. As pointed out by Dr. Strausz, current therapy for pelvic pain is unsatisfactory and the wide range of theories and the degree of operative intervention with little improvement is of concern. It was for these reasons that I felt it necessary to reassess my gynecologic skills and began incorporating neurological concepts in the evaluation of abdominal and pelvic pain.

The consistent finding of locally tender tissues in the abdominal wall, para cervix, dorsal sacrum, and levator muscles all reproducing the same chronic pain sensations strongly implicates abnormal neurological thresholds in both visceral somatic and peripheral sensory pain fibers of the T12 and S2-4 dermatomes. The association of onset with physical trauma such as that after an operation, rape, delivery, and intrauterine contraceptive device infections, etc., is commonly followed with persistence of the same pain sensations for years after resolution of the traumatic process.

The techniques of reproducing pain with (1) focal pressure (single finger, cotton tip, and/or needle tip), (2) examination of the abdominal wall by tensing the rectus muscles, and (3) blocking of tender tissues with a local anesthetic to document extended pain relief beyond the duration of action of the local anesthetic are all necessary in assessing neurological pelvic pain syndromes.

In any valuable therapy based on clinical criteria, the first step in molding the clinical approach is to gain experience under many conditions and to describe the associations of possible causes, factors, therapies, and observed outcomes in a large population.

The purpose of this paper on chronic pelvic pain thus is the description of techniques in diagnoses of what appears to be a neurologically mediated pelvic pain and the response of local infiltration. In the growing field of pain therapy these observations are consistent with those observed in other chronic pain conditions such as phantom limb, myofascitis, deafferentation syndromes, and other posttrauma chronic pain conditions.[1, 2]

Dr. Strausz questions whether the placebo effect can explain the reported successful response and to what extent "supportive therapy and medication" affected the outcome. Most of the patients seen in the pain clinic had received therapy elsewhere for the pain, 31 had hysterectomy for pain, and 61 had laparoscopy with the same pain returning after therapy (unpublished observations). In the study population all received local infiltration, 10% hysterectomy, 14% narcotics primarily for postinjection or rebound pain, 6% antidepressants, all for <1 month duration, etc. Supportive therapy then was infrequently used and only for short intervals in patients with complex clinical findings.

In a pilot study for our present double-blind saline/lidocaine/bupivacaine study, needling and operating room saline injection had no effect on pain response in six patients.

The association of psychological findings of depression, insomnia, lethargy, hypochondriases, and hysterical traits with chronic pelvic pain could as easily be a result of chronic pain as it could be a cause of chronic pain.[3] These findings are not seen in all pain patients, and when treated, the condition involves more the reaction to pain than the pain itself. These same psychological functions have been observed in other painful disease states including pelvic pain caused by pathologic diseases (endometriosis).[4]

The absence of macroscopic disease in dysmenorrhea does not exclude what we now understand is a complex neurotransmitter process of end-organ ischemia, altered thresholds with prostaglandin, vasopressin, and substance p release.[5, 6] The ability of autologous transfusions to reproduce symptoms of severe dysmenorrhea even after hysterectomy should raise serious questions about unproved but much quoted theories that primary dysmenorrhea is a result of the patient's "rejection of her role as a woman."[7]

The association of stress and emotional factors in patients with pelvic pain should not be ignored, however, because the descending control (endorphins) of the dorsal horn function in modulating threshold levels is well documented. Depression and anxiety lower the threshold, and anxiety and depression can be a result of neurochemical alterations in central nervous system function resulting from chronic pain. Psychological events resulting in stress would have the same effect of lowering thresholds but would not have to be the cause of pain in a patient with a presensitized pelvic threshold dysfunction. A Minnesota Multiphasic Personality Inventory evaluation of pain patients and control subjects with long-term follow-up is in process.

Trigger-point infiltration is not a cure but an observed clinical response to alterations in pain threshold. Much needs to be done to understand the role of pelvic pain and alterations in peripheral thresholds, including a controlled double-blind study. Let us begin by questioning these theories that have not explained the condition and by expanding our tools of evaluation.

I welcome your questions because each of us assesses pain through eyes biased by our past and honed by our experiences. Would your questions, however, be the same if you modified your examination techniques?

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False phosphatidylglycerol with Helena Fetal Tek 200 thin-layer chromatography method for fetal lung maturity

To the Editors:

We read with interest the communication by Barnes et al. (AM J OBSTET GYNECOL 1984;148:347) reporting occurrences of respiratory distress of the newborn in cases where phosphatidylglycerol had been detected in the amniotic fluids. The method of analysis was the Helena Fetal Tek 200 system, which was developed by Touchstone et al.[1] We also have utilized this procedure in routine analysis and quantified the effects of blood contamination with it.[2]

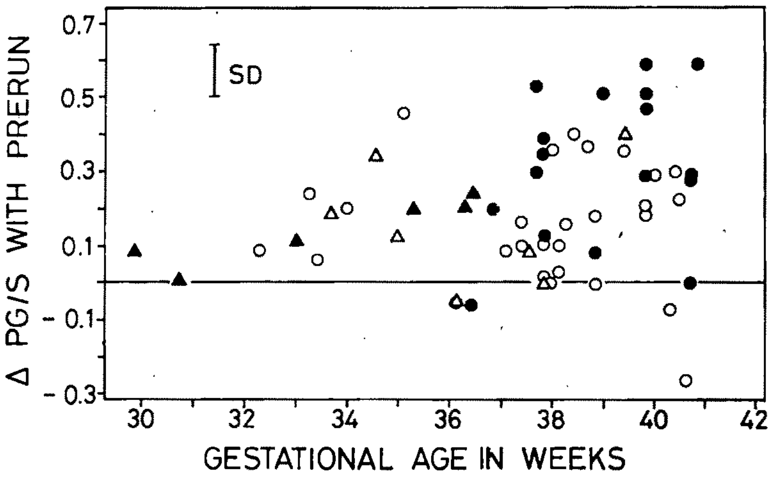

Fig. 1. Amount of pseudo-phosphatidylglycerol found in amniotic fluid, expressed as a ratio to sphingomyelin (*PG/S* ratio). The values are coded as to outcome as discussed in Reference 3, with *closed triangles* representing cases of respiratory distress syndrome; *open triangles,* mild transient difficulty; and *closed circles,* absence of any respiratory difficulty (delivery within 48 hours).

In addition, we have recognized and recently reported the presence of a substance found in most amniotic fluids which imitates phosphatidylglycerol with this system.[3] The substance ("pseudo phosphatidylglycerol"), of which Dr. Barnes and associates may have been unaware, is present in many cases in amounts greater than the genuine phosphatidylglycerol. In a sample of 75 fluids that had appeared phosphatidylglycerol-positive with the original procedure, we found 20 to be phosphatidylglycerol-negative after the removal of the contaminating substance.

The accompanying Fig. 1 shows the amount of pseudo phosphatidylglycerol we found, expressed as a ratio to sphingomyelin. Notably, the cases of respiratory distress syndrome in this population had pseudo phosphatidylglycerol as great as a ratio of 0.2, but of these cases, four were shown to actually be phosphatidylglycerol-negative after removal of the pseudo phosphatidylglycerol.

We believe that the confusion of pseudo-phosphatidylglycerol for phosphatidylglycerol with this system may be an important factor in the results that Barnes et al. report. The authors may wish to reanalyze the specimens, using the modification we have developed to eliminate the interference of "pseudo phosphatidylglycerol" in the Helena method.

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Reply to Cotton and Spillman

To the Editors:

The concept of "pseudo-phosphatidylglycerol" mentioned by Drs. Cotton and Spillman is intriguing (see Reference 3 in their letter). It remains an unidentified substance, a co-migrator with phosphatidylglycerol found resistant to digestion by *Bacillus cereus* phospholipase C. It is suggested that a prechromatography step of developing the sample on the plates for 70 minutes, equilibrating with the solvent for 10 minutes, and air-drying for 10 minutes removes pseudo-phosphatidylglycerol.

Cotton and Spillman describe a sample of 75 amniotic fluids reported initially as phosphatidylglycerol positive; however, 20 fluids (27%) were subsequently found to be, in fact, PG negative with use of the prechromatography step. We find this figure high and would expect a greater incidence of the respiratory distress syndrome with positive phosphatidylglycerol by use of our current one-dimensional thin-layer chromatography method with the Helena Tek 200 system with that percentage of error from contamination. Our experience has been that respiratory distress syndrome is rare in cases reported as phosphatidylglycerol positive. Whittle et al.,[1] in a series of patients from whom amniotic fluid had been collected within 72 hours of delivery, reported only three of 532 (0.6%) babies developed respiratory distress syndrome when phosphatidylglycerol was present.

It is of interest that it is documented[2] that the diagnostic value of phosphatidylglycerol may be valid only when the phospholipids are measured by two-dimensional thin-layer chromatography. However, Semmer et al.[3] report an analysis with the use of one-dimensional thin-layer chromatography without the prechromatography step of Cotton and Spillman. In 159 transabdominal amniotic fluid samples the phosphatidylglycerol indicated a 98% prediction rate for absence of respiratory distress syndrome with a 1.8% false positive result corrected to 0%; they concluded that the presence of >3% phosphatidylglycerol by means of Painter's[4] method of readily available one-dimensional thin-layer chromatography is consistent with fetal lung maturity. The advantages of the unidirectional method are a shorter procedure time, the ability to analyze multiple samples on a single plate, and the simplicity of a single-solvent system. Whether greater accuracy for phosphatidylglycerol interpretation is dependent on standard quantitative evaluation of phosphatidylglycerol or the removal of pseudo phosphatidylglycerol from amniotic samples may still require investigation.

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Are CA125 and placental alkaline phosphatase the same antigen?

To the Editors:

We have read with interest the paper entitled "Elevation of serum CA125 in carcinomas of the fallopian tube, endometrium, and endocervix" (Am J Obstet Gynecol 1984;148:1057), and it would appear that the monoclonal antibody OC125 has many properties similar to monoclonal antibodies directed against placental alkaline phosphatase[1] (also unpublished observations). Both CA125 and placental alkaline phosphatase are membrane-bound glycoproteins and the 'M' form of placental alkaline phosphatase has a similar molecular weight as CA125.[2] Their distribution in various tumors is similar,[3-5] although as far as we are aware placental alkaline phosphatase has not been reported in association with fallopian tube carcinoma. CA125 was initially reported as being absent from all normal human tissues, including fallopian tube, cervix, and endometrium,[6] but a later study found it to be present in these tissues[7] with a distribution identical to placental alkaline phosphatase.[8] Placental alkaline phosphatase has also been demonstrated in normal lung, testis, and thymus.[9] However, no comment was made in the later study[7] about the expression of CA125 by these organs. Both placental alkaline phosphatase and CA125 are found in association with inflammation,[1, 7] and a coelomic epithelial origin is suggested for both.

Using the monoclonal antibody NDOG$_2$, which recognizes the three common allelic forms of placental alkaline phosphatase, we have found placental alkaline phosphatase in association with 60% of endometrial carcinoma, 60% of ovarian carcinoma, and 30% of cervical carcinoma—a roughly similar distribution to CA125. Have the authors investigated the possibility that their monoclonal antibody binds a placenta-like alkaline phosphatase activity and may therefore be equated with one or many of the forms of placental alkaline phosphatase present in normal and tumor tissue?

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Reply to Davies et al.

To the Editors:

Davies and his colleagues raise an interesting question. In the early characterization of the OC125 antibody by means of indirect immunofluorescence, no binding was observed among nonmalignant tissues.[1] However, in subsequent studies using the 20-fold more sensitive biotin-avidin immunoperoxidase technique, trace amounts of CA125 have been detected in selected normal human tissues including fallopian tube, endometrium, and endocervix.[2] Although this is similar to that observed with placental alkaline phosphatase,[3] there are many differences in the tissue distributions of placental alkaline phosphatase and CA125, which suggests that they are distinct. In contrast to placental alkaline phosphatase,[4] when the biotin-avidin immunoperoxidase technique is used, CA125 is not detected in normal lung, testis, or thymus.[2] There is also a lack of OC125 reactivity with placental villi and normal ovary[2] although placental alkaline phosphatase has been consistently observed in these tissues.[4] CA125 is also not detected in decidua. Therefore, although there are some similarities in the tissue distribution of CA125 and placental alkaline phosphatase, sufficient differences are present to suggest that these are distinct determinants. Furthermore, preliminary experiments assaying for alkaline phosphatase activity in both supernatant and purified (perchloric acid soluble) antigen fractions with CA125 levels as high as 10,000 U/ml have been negative (Klug, T. L., Ph.D., personal communication).

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Ruptured corpus luteum with hemoperitoneum

To the Editors:

In their discussion of the management of ruptured corpus luteum with hemoperitoneum, Hallatt, Steele, and Snyder (AM J OBSTET GYNECOL 1984;149:5) make no mention of operative laparoscopy. In the case of a patient who is not in shock, if surgical intervention is indicated at all, laparoscopy should be primary and usually the definitive operative procedure. Laparotomy should be reserved for patients in shock from internal blood loss or for situations complicated by extensive pelvic or intraperitoneal adhesions.

A hemorrhagic corpus luteum can generally be ablated electrosurgically, by unipolar (occasionally by bipolar) techniques. Any one of a number of available laparoscopic electrosurgical "pick up" instruments is suitable for such a purpose. A cyst can be fenestrated and biopsied. The double or multiple puncture technique lends itself to such operative maneuvers. The Behrman needle (available from Elmed Inc., Addison, Illinois) is also frequently useful to stabilize the ovary. Most of the old blood of the hemoperitoneum is unclotted and readily aspirated. Fresh bleeding can be prevented from clotting by irrigation with a heparinized saline solution (for instance, 1000 U/1000 ml).

When a more extensive surgical procedure is indicated than can be achieved by laparoscopic maneuvers alone, consideration should be given to the technique of combined laparoscopy and colpotomy[1, 2] (also unpublished observations). Laparotomy should be a relatively infrequent procedure in the management of ruptured corpus luteum with hemoperitoneum.

While I have no statistical evidence to prove this, it is my strong clinical impression that laparoscopic ovarian surgery results in considerably fewer adhesions and is followed by a more rapid recovery than laparotomy. The only disadvantage of operative laparoscopic management is that concomitant appendectomy cannot ordinarily be performed.

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Reply to Freistadt

To the Editors:

There is no question about the value of laparoscopy in the diagnosis of the hemorrhagic ovary. There is considerable question as to the advisability of using the laparoscope therapeutically for hemostasis and for biopsy of ovarian pathology. The 173 cases reported were regarded as acute surgical emergencies requiring surgical hemostasis. Most hemorrhagic corpora lutea were observed or screened by laparoscopy, especially if minimal bleeding occurred as indicated by a culdocentesis fluid hematocrit value under 12%. The surgical management of the bleeding ovary by laparoscopy without a tissue diagnosis is felt to be risky because of the neoplastic potential of the ovary and the inability to accurately diagnose the ovarian pathological condition with the laparoscope. A β-human chorionic gonadotropin serum test should be done in all of the cases. The management of ectopic pregnancy and ovarian pathology via colpotomy remains a controversial subject. I am reluctant to use this approach because of the increased risk of infection in the vulnerable ovary.

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Length of stay for neonates

To the Editors:

The paper by Wright, Gardin, and Wright (Am J Obstet Gynecol 1984;149:848) provides a comparative analysis of a health maintenance organization and a fee-for-service practice. The authors report a shorter length of stay for women who received care through the health maintenance organization. However, they do not report the length of stay for neonates in the two groups. Since the incidence of prematurity is higher in the health maintenance organization group, 14.0 versus 8.4%, one might suspect that the fee-for-service neonatal patients would have shorter stays and perhaps negate the economic advantage obtained by the health maintenance organization mothers' shorter stays.

It would be valuable to know the average length of stay for the neonates. I am curious why this information was not provided.

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Reply to Hein

To the Editors:

Dr. Hein raises an interesting question that cannot be answered from our data. We did consider including certain variables of the babies, but this would have involved a great variety of pediatricians and other uncontrollable factors. Our final decision was to limit the review to those events under the control of the five obstetricians.

On the other hand, the same resources that permitted the safe, early dismissal of the mothers were available also to the babies.

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Ultrasound without history

To the Editors:

While the conclusions of O'Brien et al. (O'Brien WF, Buck DR, Nash JD. Evaluation of sonography in the initial assessment of the gynecologic patient. Am J Obstet Gynecol 1984;149:598) may be valid, it would seem that their method is flawed. it would surely seem by this time and with the evolution of medicine over the last hundred years that physicians would recognize the enormous amount of art involved in many areas of diagnostic medicine and particularly in the specific modalities of radiology and ultrasound. A protocol that involves having a sonologist read an ultrasound scan (probably done by an ultrasound technician) without the case history is like asking a radiologist to read a chest x-ray film without the history. He may be able to give you a broad description of the general nature of the chest x-ray film, but he probably provides relatively little help if you have a specific problem you are concerned about. In a similar manner a sonologist evaluating an ultrasound scan can provide the clinician with much more relevant information if he can evaluate that scan with the knowledge of the patient's history. It would seem to me almost unethical to expend medical dollars for a test that is perhaps only half performed. The fact that it was done in an Armed Forces hospital does not reduce the cost of the examination.

It is possible the results of the study might have been the same, but the foundation would certainly have been stronger if the sonologist had been given adequate patient history to aid him in interpreting the information from the scan.

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Reply to Smith

To the Editors:

We appreciate the interest of Dr. Smith in our report and would like to reply to his comments.

First, the objective of the study was the use of sonography compared to clinical evaluation by bimanual examination. We do not advocate "blinded" reading of any diagnostic examination, but this is the only way in which comparative evaluation may be accomplished.

Second, somewhat to our surprise and as reported in the results section, provision of the clinical history and physical findings to the radiologist did not result in significant improvement in the accuracy of interpretation.

Finally, as was clearly stated in the report, this investigation was a prospective funded investigation designed to answer a specific question. For a relatively low cost the results will hopefully allow considerable savings by the avoidance of unnecessary sonographic examinations.

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Books received

Alcohol and the Fetus. A Clinical Perspective. Henry L. Rosett and Lyn Weiner. 219 pages. New York, 1984, Oxford University Press. $24.95.

Atlas of Human Prenatal Histology. Edited by Hideo Nishimura. 316 pages, illustrated. New York, 1983, Igaku-Shoin Medical Publishers. $68.00.

Birth Stories. The Experience Remembered. Edited by Janet Isaacs Ashford. 189 pages, illustrated. Trumansburg, New York, 1984, The Crossing Press. $7.95 (soft cover).

Clinics in Obstetrics and Gynaecology. Volume II. Gynaecological Pathology: Advances Perspectives and Problems. H. Fox, Guest Editor. 293 pages, illustrated. Philadelphia, 1984, W. B. Saunders Company. $24.00.

Drug Treatment in Gynaecology. R. S. Ledward. 184 pages. Stoneham, Massachusetts, 1984, Butterworth Publishers. $19.95.

Embryologie Medicale. J. Langman. 411 pages, illustrated. Paris, 1984, Masson. No price listed (soft cover).

Ended Beginnings. Healing Childbearing Losses. Claudia Panuthos and Catherine Romeo. 215 pages. South Hadley, Massachusetts, 1984, Bergin & Garvey Publishers. $12.95 (soft cover).

Family Planning in Japanese Society. Samuel Coleman. 269 pages, illustrated. Princeton, New Jersey, 1983, Princeton University Press. $33.00.

Infection Control. A Policy and Procedure Manual. Monica B. Palmer. 359 pages, illustrated. Philadelphia, 1984, W. B. Saunders Company. $17.95 (soft cover).

Long-Acting Contraceptive Delivery Systems. Gerald L. Zatuchni, Alfredo Goldsmith, James D. Shelton, and John J. Sciarra. 656 pages, illustrated. Philadelphia, 1984, J. B. Lippincott Company. $25.00.

Reproduction: The New Frontier in Occupational and Environmental Health Research. James E. Lockey, Grace K. Lemasters, and William R. Keye. 628 pages. New York, 1984, Alan R. Liss, Inc. $68.00.

Spontaneous Abortion. H. J. Huisjes. 205 pages, illustrated. New York, 1984, Churchill Livingstone, Inc. $20.00.

Vascular and Doppler Ultrasound. Edited by C. Carl Jaffe. 211 pages, illustrated. New York, 1984, Churchill Livingstone, Inc. $30.00.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Changing Practice of Obstetrical Anesthesia for Normal and High Risk Pregnancy, March 21-24, 1985, Hotel Meridien, San Francisco, California. Sponsored by the University of California San Francisco, Department of Anesthesia. For further information contact: Anesthesia Research Foundation Registration Office, Attn. Sol M. Shnider, M.D., University of California S436, San Francisco, CA 94143. Tel.: (415) 666-2274.

Gynecologic Surgery—Current and Future Issues, The George Washington University School of Medicine, March 14-16, 1985. Sponsored by the Department of Obstetrics and Gynecology. For further information contact: Arnold Friedman, M.D., Department of Obstetrics and Gynecology, 2150 Pennsylvania Ave., N.W., Washington, D. C. 20037. Tel.: (202) 676-4374.

Chicago Area Medical Schools Review Course in Obstetrics and Gynecology, June 10-15, 1985, The American Congress Hotel, Chicago, Illinois. Sponsored by The University of Chicago Pritzker School of Medicine. For further information contact: CME Office, The University of Chicago, Box 139, 5841 Maryland, Chicago, IL 60637. Tel.: (312) 962-1056.

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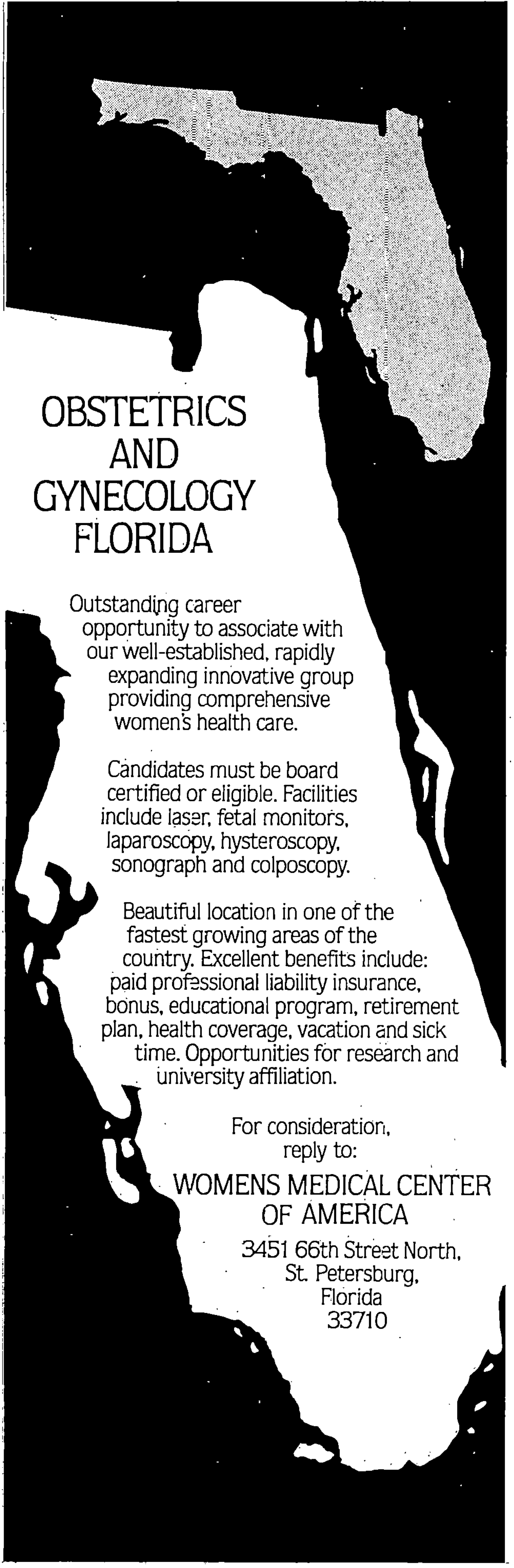

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DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use.

MYOCARDIAL INFARCTION: An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS). Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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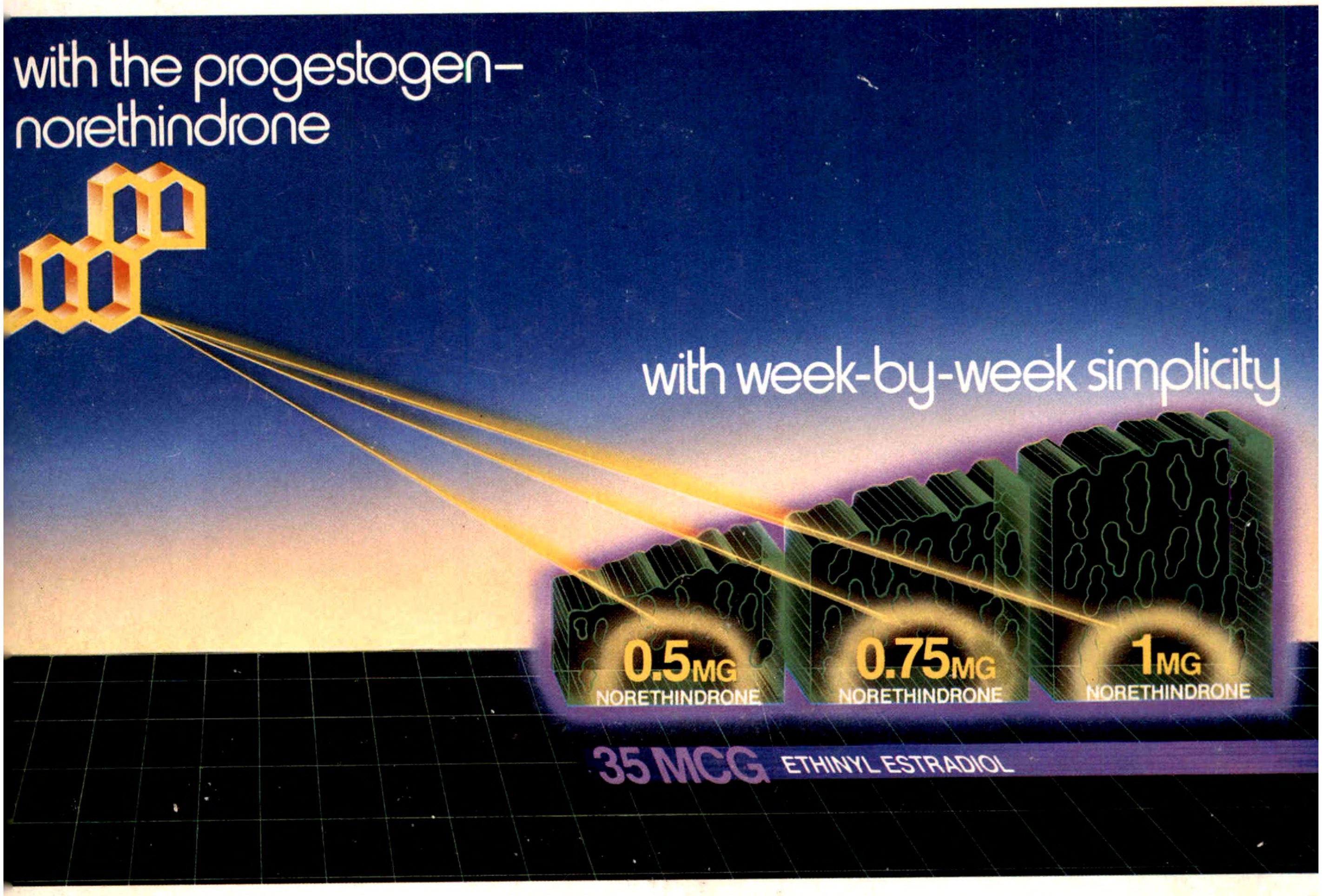

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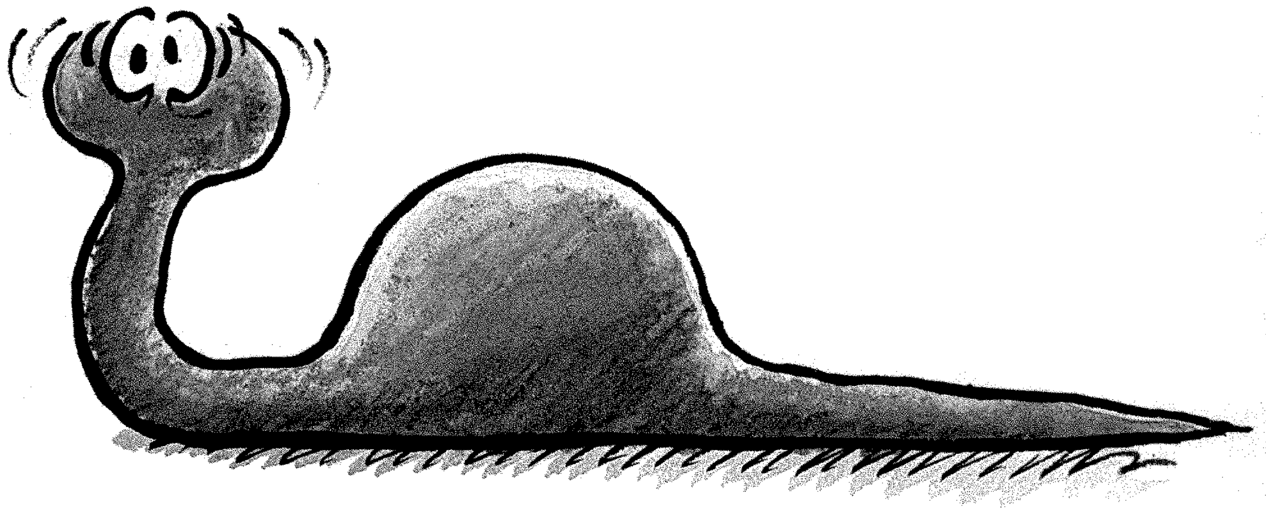

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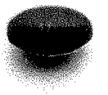

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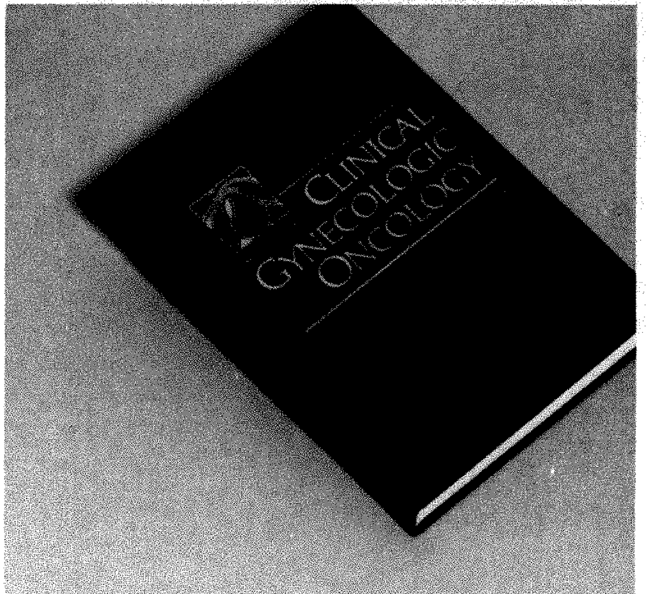

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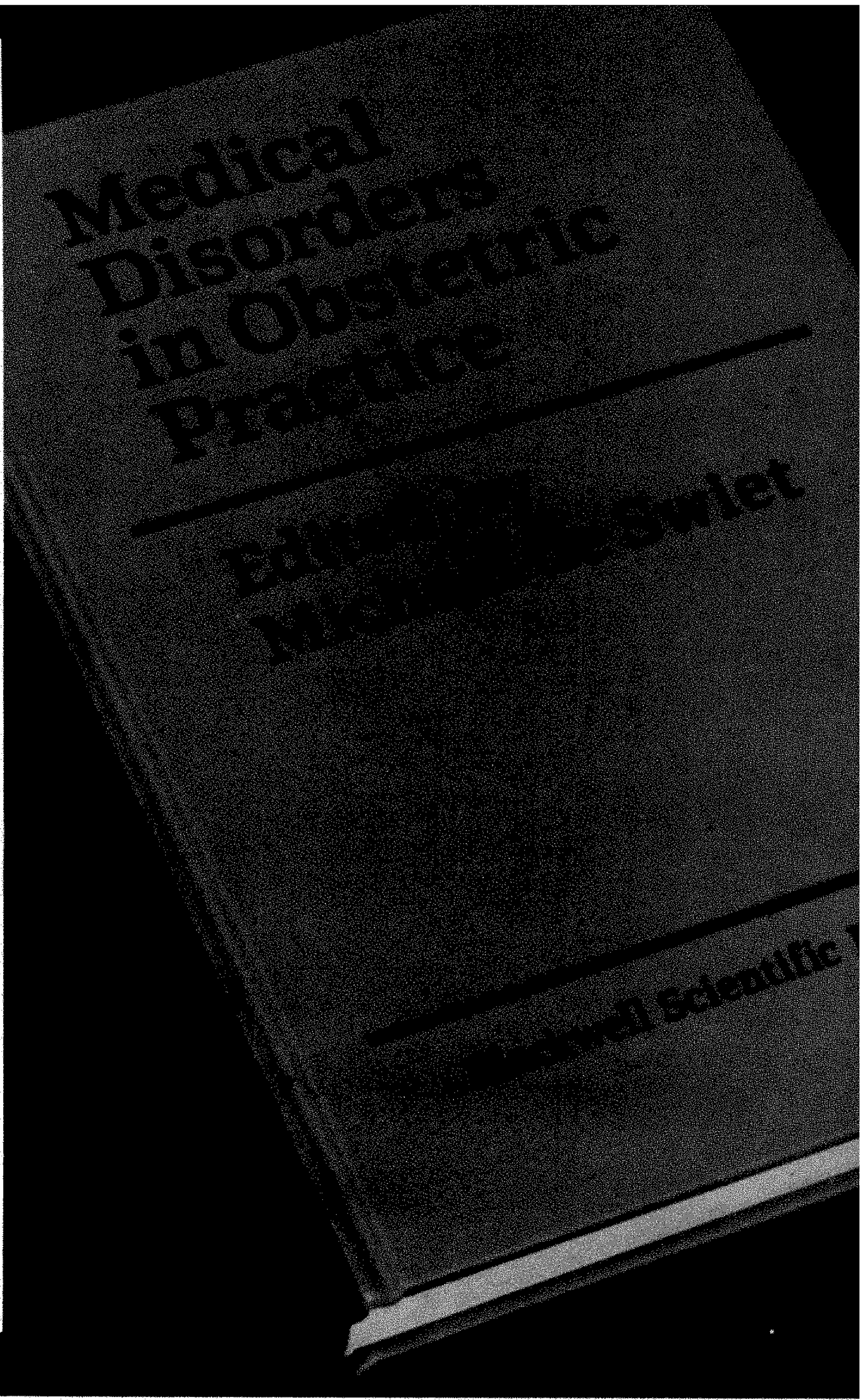
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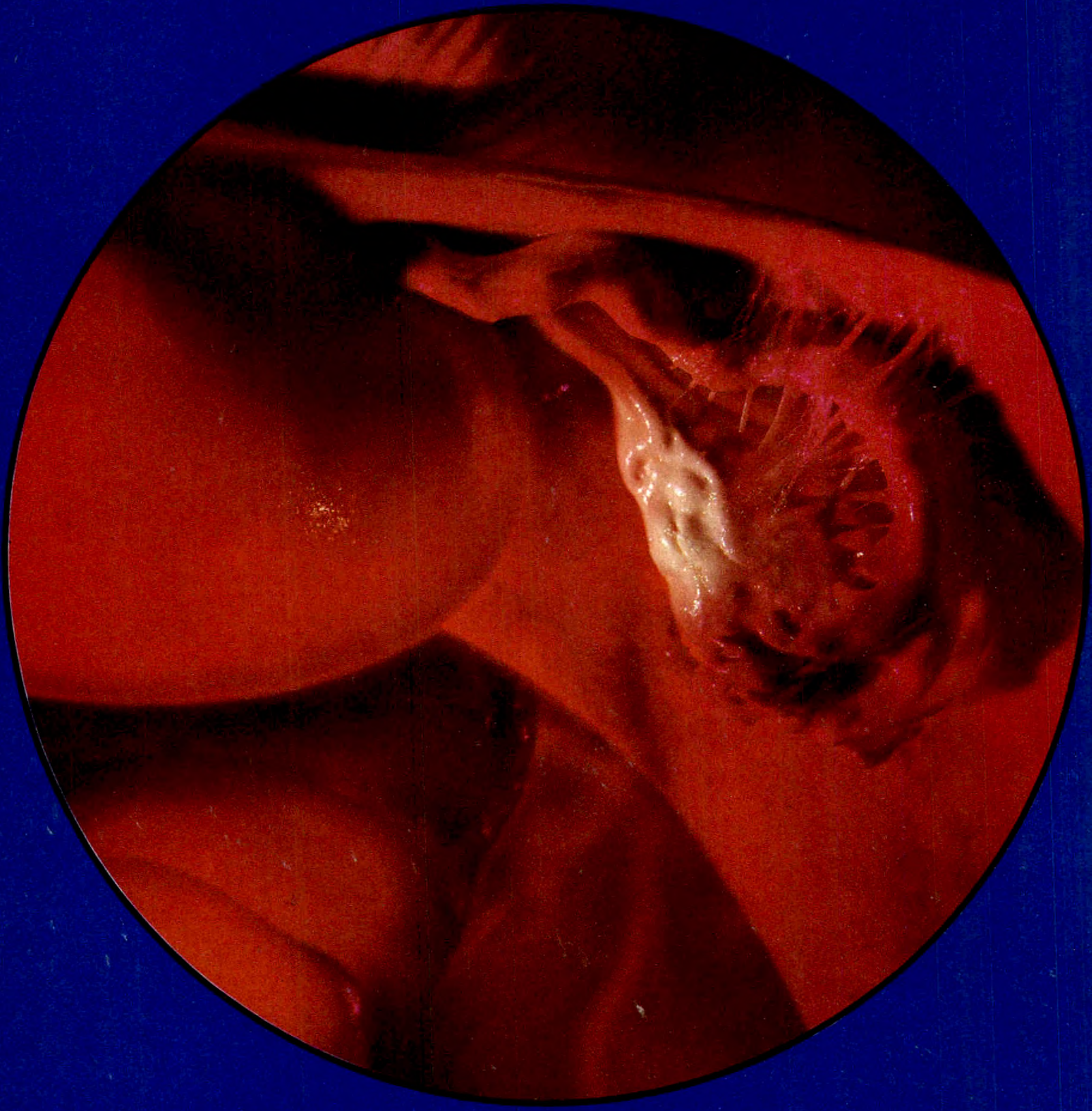

Artist's concept of the uterus
and adnexa as viewed through
a diagnostic laparoscope.
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Indications and Usage: ***Treatment***—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:
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GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).
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SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§
BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).
SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.
Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Interference with Laboratory Tests—High concentrations (>100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST‖ reagent tablets.

Pregnancy—In women of childbearing potential, weigh anticipated benefit against possible risks.

Nursing Mothers—Cefoxitin is excreted in human milk in low concentrations.

Infants and Children—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions. *Gastrointestinal*—Symptoms of pseudomembranous colitis during or after treatment and, rarely, nausea and vomiting. *Blood*—Transient eosinophilia, leukopenia, neutropenia, hemolytic anemia, and thrombocytopenia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

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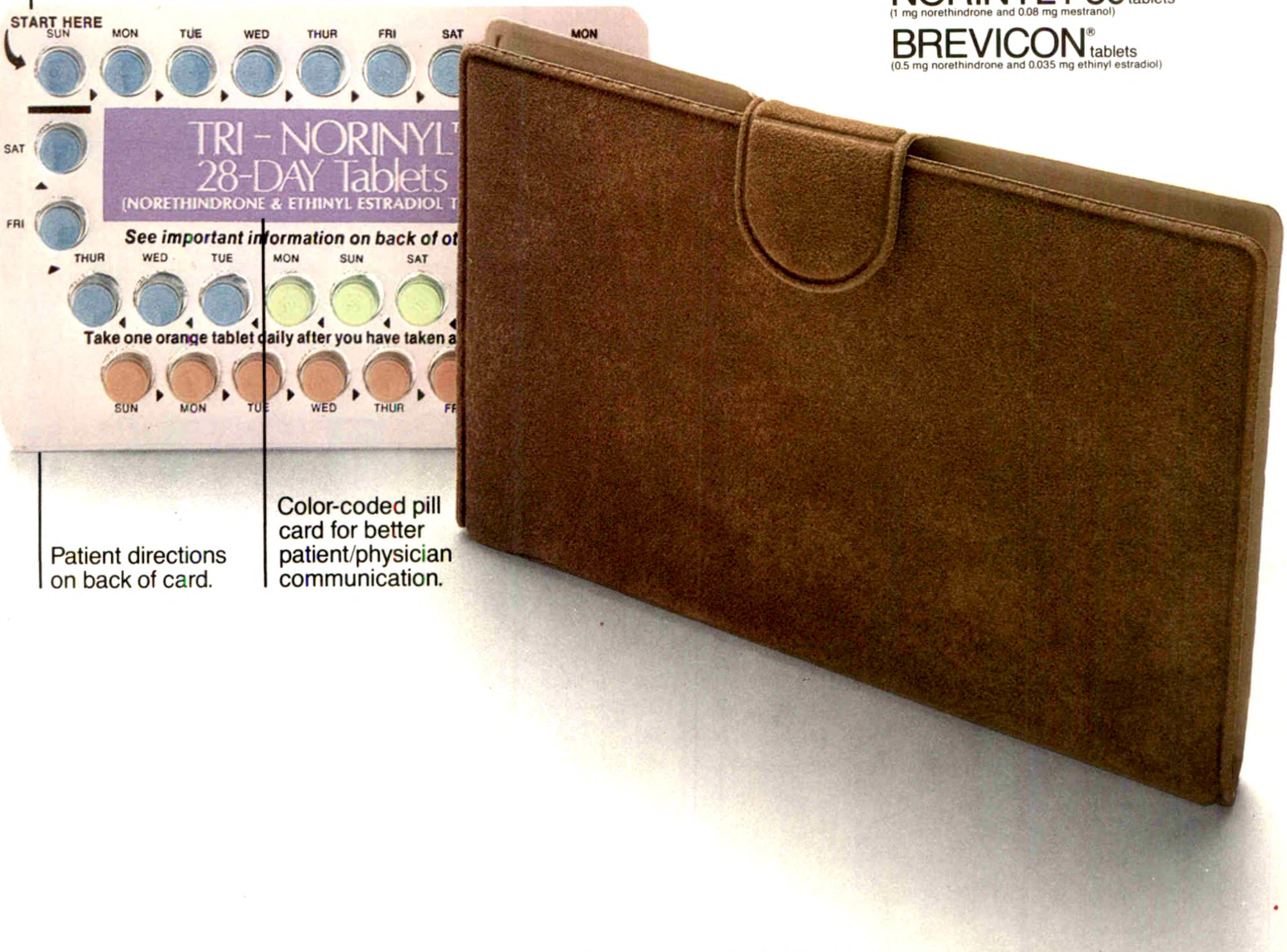
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TRI - NORINYL® 28-Day Tablets (each blue tablet contains norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, each green tablet contains norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, and orange tablets are inert)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg.)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg. tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg. with ethinyl estradiol 0.035 mg.)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg. with ethinyl estradiol 0.035 mg. tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg. with mestranol 0.05 mg.)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg. with mestranol 0.05 mg. tablets followed by 7 inert tablets)
NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg. with mestranol 0.08 mg.)
NORINYL® 1 + 80 28-Day Tablets (21 norethindrone 1 mg. with mestranol 0.08 mg. tablets followed by 7 inert tablets)
NORINYL® 2 mg. Tablets (norethindrone 2 mg. with mestranol 0.1 mg.)
NOR-Q.D.® (norethindrone) Tablets 0.35 mg.

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications: 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as [illegible] times greater in users than nonusers. A British long-term follow-up study reported in [illegible] a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone. MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. *Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.*

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk.
B—Use associated with high risk.
C—Use associated with moderate risk.
D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosised persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OC's with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic anti depressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Contraindications and Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hermorrhagic eruption, vaginitis, porpyhria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

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Respiratory sinus arrhythmia in the human fetus

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This study explores the relationship between fetal heart rate variability and fetal breathing movements in the term fetus. Fourier analysis was applied to fetal heart rate variability during fetal breathing and nonbreathing episodes. A distinct component in the order of 0.7 to 0.95 Hz was demonstrated in fetal heart rate variability during breathing episodes. These frequencies have the same distribution as the corresponding rate of breathing and therefore indicate that respiratory sinus arrhythmia exists in the term fetus. (AM J OBSTET GYNECOL 1985;151:425-8.)

Key words: Fetal heart rate, fetal breathing, respiratory sinus arrhythmia

The assessment of fetal heart rate variability and its significance to fetal well-being have received increased attention in recent years.[1] Variations in heart rate are attributed to modulation of the depolarization rate of the sinoatrial node by a number of physiologic mechanisms such as blood pressure control, thermoregulation, and respiration.[2]

Respiratory sinus arrhythmia is the phenomenon by which respiration influences heart rate in the human.[3] This was proved by utilizing Fourier analysis techniques to analyze the interaction between breathing and heart rate in the neonate[4] and the adult.[5] The results showed that the respiratory waveform and the corresponding instantaneous heart rate had a common frequency fluctuation.

Previous studies that examined the association between changes in fetal heart rate and fetal breathing movements showed that fetal heart rate variability increased during breathing episodes.[6] This increased fetal heart rate variability is thought to be a nonrandom process. It has also been associated with uterine contractions,[7] mild hypoxemia,[8] mild acidemia,[9] and fetal active states.[10] The purpose of this study was to determine whether respiratory sinus arrhythmia exists in the term fetus by applying Fourier analysis techniques to fetal heart rate variability in a manner that would show components of fetal heart rate variability that are frequency related to the rate of fetal breathing movements.

Material and methods

Patients. Eighteen healthy women at 38 to 41 weeks of gestation were selected for this study. They all gave their informed consent. They had no complications of pregnancy and had no history of smoking or use of any medications (other than ferrous compounds and/or vitamins). The study was performed after spontaneous rupture of membranes and before the onset of uterine contractions.

Data input. The fetal electrocardiograms were obtained via a scalp electrode and a fetal monitor (Hewlett-Packard, 8030A). Fetal activity was concomitantly monitored by an observer using a real-time ultrasound scanner (ADR-Ultrasound, 2130, transducer 3.5 mHz). Two event markers were used to record fetal breathing and body movements. A similar system was compared by Cousin et al.[11] and Adamson et al.[12] to an automatic tracking system for detection of fetal breathing movements. They concluded that with the use of real-time scanners, coupled with observer-operated event markers, it was possible to reliably quantitate the rate and incidence of these movements.

All data inputs (i.e., fetal electrocardiogram, breathing, gross body movements) were simultaneously recorded on three channels of a four-channel FM tape recorder (TEAC Instrumentation, R-60) for a period of 25 minutes. On replay the signals were fed into a microcomputer (Compucolor II, 32K memory) with a sampling rate of 0.1 msec.

Data selection. An 8-minute study period was se-

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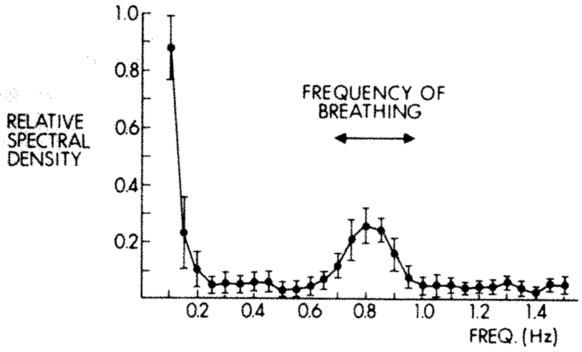

Fig. 1. Mean spectral densities (±SEM) of beat-to-beat variability during fetal breathing episodes. The *double-headed arrow* indicates the frequency of fetal breathing (mean ± SD).

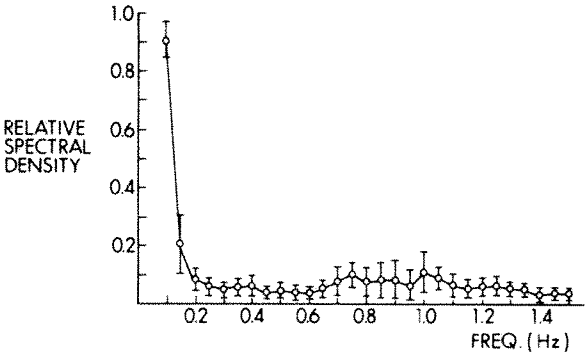

Fig. 2. Mean spectral densities (±SEM) of beat-to-beat variability during "no breathing" episodes.

lected from each patient for further evaluation. Selection was arbitrarily comprised of the first four 1-minute intervals that contained continuous fetal breathing and the first four 1-minute intervals that contained neither fetal breathing nor fetal body movements. Apnea was defined as absence of fetal breathing movements for >6 seconds.

Data processing. Beat-to-beat intervals were derived from the fetal electrocardiogram signal by detecting the time interval between each consecutive R-wave peak. Beat-to-beat variability was then continuously calculated as $[Tn - T(n - 1)]/[Tn + T(n - 1)]$ where Tn = the n*th* interbeat interval.[13] Breathing rate was calculated from breath-to-breath intervals and expressed as a mean ± SD (in breaths per minute).

Spectral density calculations. The original R-waves occur at irregular intervals whereas the analysis technique used herein needs equal interval samples of the beat-to-beat variability function. This was achieved by fitting the experimental data with a first-order polynomial and then sampling this polynomial at equal intervals of time. Plots of frequency spectra at 0.1 to 1.5 Hz were obtained by means of a conventional Fourier transform algorithm.[14] The sampling rate was chosen to satisfy the Nyquist requirement, and the signals were filtered to prevent aliasing.[15]

Statistical methods. The estimates of spectral densities of beat-to-beat variability during breathing episodes were pooled for all patients ($n = 60$). Estimates of all nonbreathing episodes were similarly pooled ($n = 60$). Segments of 0.05 Hz increments of the total frequency spectra were used to calculate mean spectral densities (±SEM) for each of the two states. Student's *t* test was used to compare the pooled values for breathing and nonbreathing periods.

Results

Fetal outcome. The outcome of each of the pregnancies was entirely normal. All patients delivered vaginally with Apgar scores ≥ 8 at 1 and 5 minutes. Gestational age was confirmed by neonatal examination. Birth weights were appropriate for gestational age.

Fetal breathing. Four recordings containing continuous breathing for 1 minute were obtained from 15 of the 18 patients participating in the study. The mean breathing rate for these 60 samples was 49 ± 8 (±SD) breaths per minute with a range of 37 to 63 breaths per minute (0.61 Hz to 1.05 Hz).

Power spectrum of beat-to-beat variability. Figs. 1 and 2 show the mean (±SEM) spectral densities obtained from 15 fetuses during episodes of breathing and nonbreathing respectively. The spectral density was significantly higher at the lower frequencies for both states. Differences between states were not significant. A component of 0.2 to 0.65 Hz was weak or negligible for both states. Significantly dominant peaks were found between 0.7 and 0.95 Hz during fetal breathing ($p < 0.01$). The frequency distribution of these peaks was similar to the rate of breathing (Fig. 1).

Comments

The relationship between human fetal breathing movements and a change in the patterns of fetal heart rate was noted, but not quantified, by Timor-Trisch et al.[16] Several investigators used statistical methods to describe the global variations in beat-to-beat variability during fetal breathing. Wheeler et al.[17] found an increase in both short- and long-term variability during breathing episodes. Dawes et al.[6] examined the fetal heart rate via abdominal wall fetal electrocardiograms in the presence of fetal breathing and found a mean increase of 1 msec in the short-term beat-to-beat variability, while the mean fetal heart rate fell by 2.8 bpm. These statistical methods tend to destroy the temporal sequence of the individual interbeat intervals and therefore were not suitable for this study.

Fourier analysis is an alternative method, since it is a frequency domain technique that is commonly used to identify rhythmic variations in a record of physiologic events by decomposing it into its basic frequency components. It has previously been used to document respiratory sinus arrhythmia in the term neonate[1] and was chosen for this study because it was likely there would be similarities in the control of heart rate variability between the newborn infant and the healthy term fetus.

Fourier analysis of beat-to-beat variability was performed on 60 1-minute breathing samples and 60 1-minute nonbreathing samples. The significant finding in this study is that consistent frequencies (from 0.7 to 0.95 Hz) are present in beat-to-beat variability during breathing episodes. These frequencies have the same distribution as the corresponding rate of breathing (Fig. 1) and therefore indicate that respiratory sinus arrhythmia exists in utero and is not a consequence of the major physiologic changes that accompany birth.

The mean spectral densities of beat-to-beat variability during nonbreathing episodes were slightly elevated between 0.7 and 1.05 Hz (Fig. 2). Possible explanations for this increase include the following: (1) Some fetal breathing movements might be too slight to be visually recognized.[12] (2) Rhythmic breathing movements are not an absolute requirement for the appearance of respiratory sinus arrhythmia. For example, a modulation of heart rate similar in time course may be seen at the onset of an inspiratory breath hold and in animal experiments in which respiratory movements were blocked by curarization.[18]

The effect of respiration on heart rate respiratory sinus arrhythmia has long been recognized in extrauterine life. Many explanations have been proposed. It has been considered as a lung inflation reflex.[19] Others have suggested that its origin is within the central nervous system and that it is due to interaction between the respiratory centers and the autonomic control of heart rate.[20] Alternatively, increased filling of the right atrium during inspiration has been suggested as a cause of cardiac acceleration.[21] Recent studies of respiratory sinus arrhythmia[20, 22] are in agreement that the cause of respiratory sinus arrhythmia is central modulation of the baroreceptor reflex.

Both fetal breathing and fetal heart rate variability are commonly used for evaluation of fetal well-being.[23] Power spectrum analysis techniques have provided the means for quantifying the interaction between these biophysical variables. A combined rather than a separate analysis may prove useful because of the fact that biophysical activities are initiated and regulated by complex, integrated central nervous system mechanisms.[23] Also of significance to this concept is the fact that nervous tissue is the most oxygen-dependent tissue and that modulation of fetal heart rate by breathing could provide an indirect measure of an intact and functioning autonomic nervous system. Indeed, it has been previously used to detect brain stem dysfunction in the infant[24] and the adult.[25]

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Biochemical and morphologic effects of ethanol on fetuses from normally ovulating and superovulated mice

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To further our understanding about the teratogenicity of ethanol, we studied the changes produced by this compound in the incorporation of glucose ^{14}C into several tissue fractions of 12-day mice fetuses and the morphologic alterations present at day 19 of gestation. Furthermore, to determine if the fetal toxicity of ethanol was modified by changes in the characteristics of the pregnancy, we performed similar biochemical and morphologic observations in fetuses from superovulated mice. Maternal ethanol administration caused biochemical alterations in the fetal tissues of both normally ovulating and superovulated mice, but it caused morphologic alterations predominantly in fetuses from superovulated animals. The prevalent morphologic alteration found in ethanol-treated animals was a hemorrhagic lesion dorsal to the lumbar or dorsal vertebrae. These results suggest that superovulation increases the susceptibility of the fetal mice tissues to the teratogenic effects of ethanol. (AM J OBSTET GYNECOL 1985;151:428-33.)

Key words: Ethanol, teratogenicity, superovulation

There is considerable evidence indicating that alcohol ingestion during pregnancy has fetotoxic effects. In humans a fetal alcohol syndrome has been found in the offspring of mothers who had moderate to heavy alcohol consumption during pregnancy.[1-6] This syndrome is characterized by a constellation of facial features (short palpebral fissures, epicanthic folds, short nose, low nasal bridge, narrow upper lip, small chin), by growth retardation, and by psychomotor disturbances. In experimental animals, alcohol administration during pregnancy has failed to produce a characteristic morphologic defect, although reduced litter and embryo size as well as multiple malformations have been described.[7-11]

The development of clinical knowledge about the fetal toxicity of ethanol has not been paralleled by advances in understanding the mechanisms leading to the production of malformations. In an effort to better understand these mechanisms, we have analyzed some of the biochemical changes acutely produced by ethanol in 12-day mice fetuses and attempted to correlate these changes with the presence of morphologic alterations at the end of pregnancy. Then, to determine whether or not the effects of ethanol are modified by changes in the intrauterine environment, we performed similar studies in superovulated mice.

Material and methods

Materials. Pregnant mare serum gonadotropin, human chorionic gonadotropin, calf thymus DNA, ribonuclease A (E.C. No. 3.1.4.22), actinomycin D, and diphenylamine were obtained from Sigma Chemical Company, St. Louis. Progesterone (pregn-4-ene-3,20-dione) was obtained from Steraloids, Inc., Wilton, New Hampshire. D-U glucose ^{14}C (specific activity, 230 mCi/mmol) was obtained from Amersham, Arlington Heights, Illinois. Amyloglucosidase (1,4-α-D-glucan glucohydrolase, E.C. No. 3.2.1.3) was obtained from Boehringer Mannheim, Indianapolis, Indiana.

Animals. Female ICR mice (Harlan Sprague-Dawley, Indianapolis, Indiana), 6 to 10 weeks of age, were housed five per cage, placed in a room with a 12-hour light/12-hour dark cycle, and fed with Purina Labo-

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Table I. Metabolic effects* of superovulation on 12-day mice fetuses

	Normally ovulating mice	*Superovulated mice*
Litters (n)	9	20
Lipids	10.6 ± 1.6	27.3 ± 6.6†
RNA	3.8 ± 1.1	13.9 ± 3.7†
Glycosaminoglycan	2.2 ± 0.5	8.2 ± 2.8†
Carbohydrate	1.2 ± 0.2	3.7 ± 2.0†
DNA	5.3 ± 1.0	10.3 ± 2.8†
Protein	12.8 ± 1.5	32.5 ± 13.1†

*Expressed as counts per minute of ^{14}C incorporated in each fraction per microgram of DNA ± SD.

†Significant difference ($p < 0.01$).

Table II. Morphologic effects of superovulation on 19-day mice fetuses

	Normally ovulating mice	*Superovulated mice*
Litters	21	17
Implants	218 (10.3†)	509* (29.9†)
Fetuses	205 (9.7†)	402* (23.6†)
Fetal weight (gm)	1.166 ±0.013	0.700* ±0.16
Resorptions	13 (5.9%)	107* (21.0%)
Fetuses with intrauterine growth retardation	8 (3.9%)	257* (63.9%)
Fetuses with abnormalities	1 (0.4%)	146* (35.2%)

*Significantly different ($p < 0.01$).

†Number indicates mean occurrence of the event per animal.

Table III. Metabolic effects* of ethanol on 12-day fetuses from normally ovulating mice

	Control mice	*Ethanol-treated mice*
Litters	9	9
Lipids	10.6 ± 1.6	39.6 ± 16.3†
RNA	3.8 ± 1.1	7.4 ± 1.7†
Glycosaminoglycan	2.2 ± 0.5	10.2 ± 4.0†
Carbohydrate	1.2 ± 0.2	3.9 ± 1.9†
DNA	5.3 ± 1.0	7.2 ± 2.8
Protein	12.8 ± 1.5	31.8 ± 10.2†

*Expressed as counts per minute of ^{14}C incorporated in each fraction per microgram of DNA ± SD.

†Significantly different from the control group ($p < 0.01$).

Table IV. Morphologic effects of ethanol on 19-day fetuses from normally ovulating mice

	Control mice	*Ethanol-treated mice*
Litters	21	18
Implants	218 (10.3*)	164 (10.2*)
Fetuses	205 (9.7*)	151 (9.4*)
Fetal weight (gm)	1.166 ±0.13	1.128 ±0.18
Resorptions	13 (5.9%)	13 (7.9%)
Fetuses with intrauterine growth retardation	8 (3.9%)	9 (5.9%)
Fetuses with abnormalities	1 (0.4%)	5† (3.2%)

*Number indicates mean occurrence of the event per animal.

†($p < 0.01$).

ratory Chow and tap water. After a minimum of 7 days of adaptation to the light/dark cycles, each female was placed in the same cage with a fertile male and examined daily for the presence of a vaginal plug. The day a vaginal plug was detected was designated as day 1 of the pregnancy.

For induction of superovulation, female mice were injected intraperitoneally with 5 U of pregnant mare serum gonadotropin and, 44 hours later, with 5 U of human chorionic gonadotropin. The night following the injection of human chorionic gonadotropin the female was placed with a fertile male and the following morning examined for the presence of a vaginal plug. If a vaginal plug was found, that was considered to be day 1 of the pregnancy. If no vaginal plug was present, the animal was removed from the male's cage and not used for further experiments.

Animal treatment protocols. On day 12 of pregnancy, which is comparable to day 36 of human gestation,[12] each group of pregnant mice (superovulated or not) was subdivided into the following treatment subgroups: (1) *Ethanol-treated animals.* These mice were injected subcutaneously with 0.01 ml of ethanol per gram of body weight, with use of a 50% (v/v) solution of absolute ethyl alcohol in 0.9% saline solution. (2) *Control animals.* These mice were injected subcutaneously with 0.9% saline solution, with use of a total volume equal to that required by the ethanol-treated animals.

Two hours after injection, one half of the animals in each subgroup received intravenously (lateral tail vein) 0.5 mCi of glucose ^{14}C (specific activity, 230 mCi/mmol/kg). Three hours later the animals were sacrificed by cervical dislocation, the pregnant uteri were removed, and the fetuses isolated and treated as described below.

The other half of the animals in each subgroup were sacrificed on day 19 of gestation. The pregnant uteri were removed, and the number of implants, live fetuses, and resorptions were recorded. Each fetus was weighed and examined for the presence of external

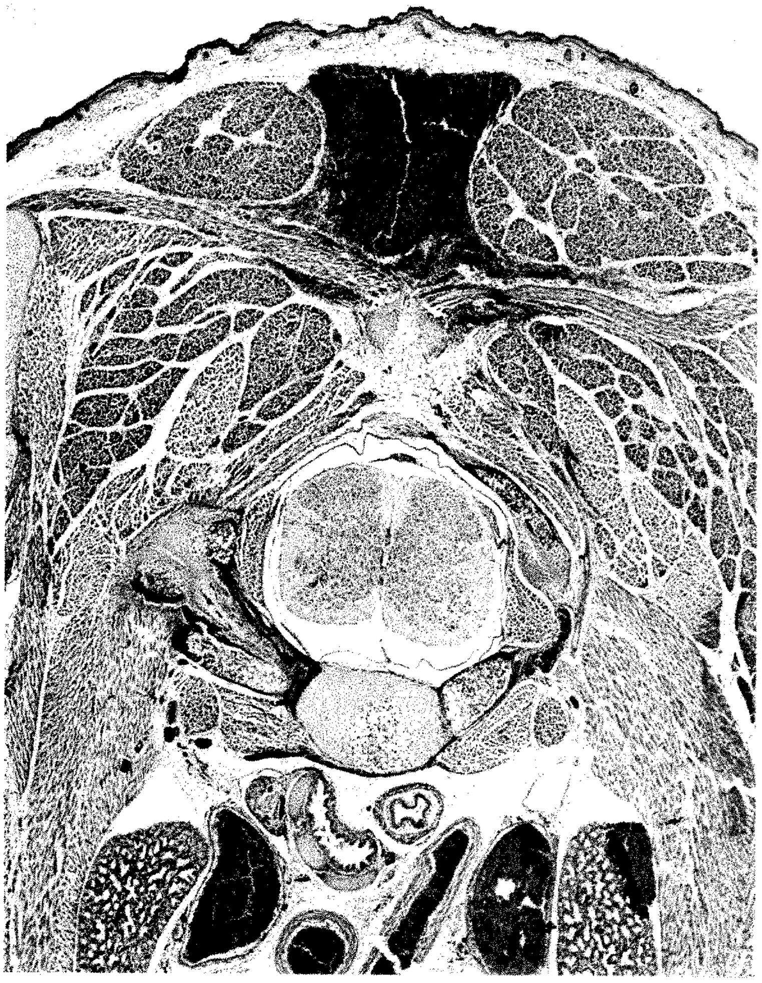

Fig. 1. Microscopic appearance of the hemorrhagic lesion frequently found in ethanol-treated mice. This section was made in the low dorsal region and shows a large hemorrhage between the posterior aspect of the spine and the skin.

malformations, and after fixation in formalin, serial transverse sections made with a razor blade through the head, thorax, and abdomen were also analyzed.[13]

For the purpose of evaluating the outcome of pregnancy, intrauterine growth retardation was defined as birth weight ≥2 SD below the mean weight of the 19-day fetuses obtained from control mice pregnant after normal ovulation.

Preparation of fetal subcellular fractions. Fetuses with normal morphologic features were collected 5 hours after ethanol or saline solution administration and 3 hours after injection of the "nonspecific" radioactive precursor and were deposited in cold 0.2N perchloric acid. The fetuses pooled from an entire litter were homogenized by means of a Potter-Elvehjem homogenizer. This material was centrifuged at 2750 × g and 4° C for 15 minutes. The supernatant was discarded, and further fractionation of the pellet was carried out following the methodology described by Krowke et al.[14] Briefly, the method consists of repeated ethanol/ether extractions to obtain the lipid fraction, followed by digestion with ribonuclease (100 μg per ml of 0.1 mol/L of Tris-hydrochloride buffer, pH 7.5) to separate the RNA fraction, digestion with 0.3N sodium hydroxide for 1 hour at 37° C to obtain the glycosaminoglycan fraction, digestion with amyloglucosidase (1 mg per ml of 0.1 mol/L of sodium acetate buffer, pH 4.9) to obtain the carbohydrate fraction, and digestion with 0.5N perchloric acid to separate the DNA and protein fractions. The amount of radioactivity present in each of these fractions was determined by adding an aliquot to liquid scintillation fluid and measuring the radioactivity in a model LS 330 Beckman scintillation counter. The incorporation of the isotope in each subcellular fraction was calculated and expressed as counts per minute per microgram of DNA. DNA was quantified with use of the diphenylamine method[15] with calf thymus DNA as standard. Ethanol tissue concentrations were measured according to the procedure described by Bernt and Gutmann.[16]

The statistical significance of the biochemical and morphologic differences found among fetuses belonging to each of the different subgroups was calculated with the Student's *t* test and the χ^2 test.

Results

Metabolic and morphologic effects of superovulation. When animals injected with saline were compared, it was found that fetuses from superovulated mice had significantly greater ^{14}C incorporation into all different biochemically biochemical fractions than fetuses from normally ovulating mice (Table I). Furthermore, significant differences in the outcome of pregnancy were found between animals pregnant after normal ovulation and superovulated animals. The number of implants, resorptions, and fetuses and the instances of growth retardation and abnormalities were significantly greater in superovulated animals. The mean fetal weight was significantly smaller in superovulated animals (Table II).

Metabolic and morphologic effects of ethanol on fetuses from normally ovulating mice. As shown in Table III, ethanol produced a significant increase in ^{14}C incorporation into all fetal subcellular fractions, except the DNA. With respect to the number of implants, number of fetuses, and mean fetal weight, no significant differences were found between control and ethanol-treated fetuses. Also, no significant differences were found among ethanol-treated and untreated animals with respect to the frequency of resorptions and the incidence of fetuses with intrauterine growth retardation (Table IV). The ethanol concentration in the fetal tissues was 7.6 ± 1.02 μmol per gram.

With respect to specific malformations, two out of 151 fetuses (1.9%) in the ethanol-treated group exhibited severe developmental abnormalities and consisted of a mass of poorly differentiated tissue attached to a small but well-defined placenta, similar to what Bernt and Gutmann[16] have described as severe growth disorganization. The other three abnormal fetuses in the ethanol group presented a large hemorrhage dorsal to the lumbar or dorsal vertebrae with the characteristics of a subcutaneous hematoma (Fig. 1). No hemorrhages were seen in fetuses from the control group. No visceral malformations were found in the ethanol-treated group.

Metabolic and morphologic effects of ethanol in fetuses from superovulated mice. Fetuses from ethanol-treated animals showed significantly greater ^{14}C incorporation than the control animals into all the biochemical fractions under study, except the glycosaminoglycan fraction (Table V). The ethanol concentration in fetal tissues of superovulated animals was 5.3 ± 1.7 μmol per gram, a value that was not significantly different from that obtained in normally ovulating animals.

Table V. Metabolic effects* of ethanol on 12-day fetuses from superovulated mice

	Superovulated control mice	*Ethanol-treated mice*
Litters	20	9
Lipids	27.3 ± 6.6	44.0 ± 15.3**
RNA	13.9 ± 3.7	32.0 ± 14.1**
Glycosaminoglycan	8.2 ± 2.8	8.4 ± 4.1**
Carbohydrate	3.7 ± 2.0	6.4 ± 4.0**
DNA	10.3 ± 2.8	15.3 ± 3.5**
Protein	32.5 ± 13.1	69.6 ± 16.0**

*Expressed as counts per minute of ^{14}C incorporated in each fraction per microgram of DNA ± SD.

**Significantly different from the superovulated control group ($p < 0.01$).

Superovulated animals treated with ethanol exhibited several significant differences from the superovulated control group: the ethanol-treated group had significantly fewer implants and fetuses and significantly more fetuses with abnormalities (Table VI). Forty-nine of the fetuses (20.16%) in the ethanol-treated group exhibited hemorrhagic lesions behind the spine, and in one half of these, there was a concomitant defect in the overlying soft tissues (Fig. 2). In contrast, this lesion was present in only 9.95% of the superovulated control fetuses, and none of these had the associated skin defect.

Comment

As expected, we found that superovulation caused a significant increase in the number of implants and fetuses and in the incidence of intrauterine growth retardation. However, it also caused a significant increase in the number of resorptions and in the number of abnormal fetuses. The latter fact suggests that treatment with gonadotropins may increase the frequency of genetically abnormal eggs or may increase the susceptibility of the fetal tissues to multifactorial influences. Some investigations[17, 18] are consistent with these possibilities, although it is fair to say that most of the literature does not support the existence of a cause-effect relationship between ovulation induction and birth defects.[19]

Administration of ethanol to mice pregnant after normal ovulation caused a significant increase in the incorporation of the nonspecific radioactive precursor into all fetal tissue fractions except the DNA, when compared with normally ovulating control mice injected with saline solution. Surprisingly, these marked

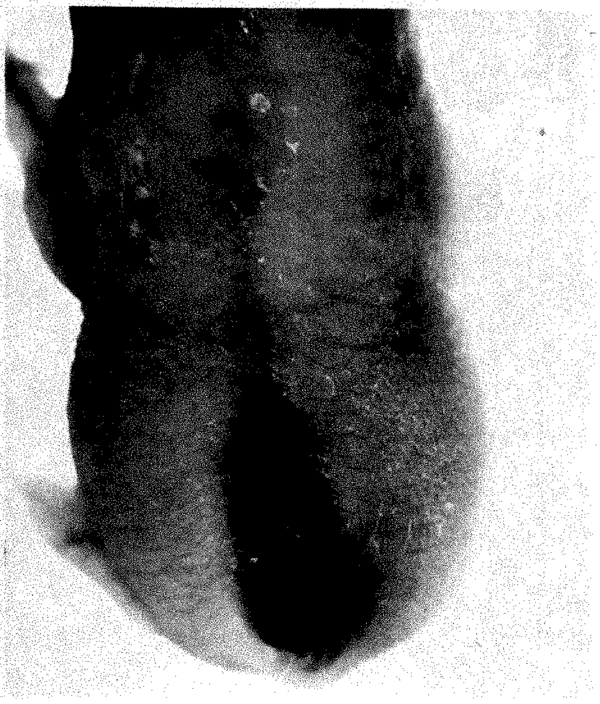

Fig. 2. Macroscopic appearance of a severe hemorrhagic lesion frequently found in ethanol-treated, superovulated mice. Here the hematoma has opened to the exterior, exposing the lumbar spine.

Table VI. Morphologic effects of ethanol on 19-day mice fetuses conceived after superovulation

	Superovulated control mice	*Ethanol-treated mice*
Litters	17	19
Implants	509 (29.9†)	300* (15.1†)
Fetuses	402 (23.6†)	243* (12.7†)
Fetal weight (gm)	0.700 ±0.16	0.665 ±0.26
Resorptions	107 (21.0%)	44 (15.3%)
Fetuses with intrauterine growth retardation	257 (63.9%)	175* (73.2%)
Fetuses with abnormalities	146 (35.2%)	130* (53.4%)

*Significantly different from the control group ($p < 0.01$).

†Number indicates mean occurrence of the event per animal.

changes in fetal metabolism did not result in significant differences with the control mice with respect to fetal morphology at the end of gestation. The discrepancy between metabolic effects and morphologic changes probably means that under our experimental conditions the metabolic insult induced by ethanol was not large enough, prolonged enough, or repeated often enough to cause malformations.

We found significant differences between fetuses from superovulated mice injected with ethanol and fetuses from superovulated control animals treated with saline solution. In fact, administration of ethanol resulted not only in a significant increase in radioactive precursor incorporation into all subcellular fractions, but also in a significant increase in the number of abnormal fetuses found at the end of gestation. Since the fetal tissue alcohol concentration was similar in normally ovulating and superovulated mice, our results suggest that the metabolic and morphologic effects of ethanol upon the fetus are magnified when it is administered to mice pregnant after superovulation. It is possible (as suggested when normally ovulating and superovulated control mice are compared) that superovulation increases the susceptibility of the fetal tissues to teratogenic agents and allows a more complete expression of both the metabolic and the morphologic effects of ethanol on the fetus.

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Plasma and red blood cell β-endorphin immunoreactivity in normal and complicated pregnancies: Gestational age variation

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Recent observations suggest that there may be two pools of β-endorphin–like immunoreactivity in mammalian circulation. One of these pools is present in plasma and the other is detected in association with erythrocytes. Elucidation of an erythrocyte-associated pool may explain some of the wide variability of plasma β-endorphin levels reported in the literature. We measured β-endorphin immunoreactivity levels in 85 normal and 33 complicated pregnancies to delineate a possible correlation between gestational age and β-endorphin immunoreactivity levels in plasma and in erythrocytes. Our results indicate that β-endorphin immunoreactivity levels in both plasma and erythrocytes vary systematically throughout the gestational period, reaching a peak at 31 to 32 weeks of gestation. Amniotic fluids at midgestation were also analyzed and no correlation was observed between the levels of β-endorphin immunoreactivity and fetal sex. Compared to normal patients, diabetic patients had significantly lower levels of β-endorphin immunoreactivity in plasma and higher levels in erythrocytes although the total β-endorphin immunoreactivity was not statistically different from that in normal subjects. We conclude that (1) the total β-endorphin immunoreactivity level in whole blood is much higher than that reported in plasma, (2) both plasma- and erythrocyte-associated β-endorphin immunoreactivity levels vary with gestational age, with a peak level at 24 to 32 weeks of gestation, (3) amniotic fluid β-endorphin immunoreactivity levels are unrelated to fetal sex, and (4) diabetic patients may have a different distribution of β-endorphin immunoreactivity pools than normal individuals. (AM J OBSTET GYNECOL 1985;151:433-7.)

Key words: β-Endorphin, pregnancy, erythrocytes, gestation

Maternal physiologic adaptation to pregnancy has been studied extensively and alterations in many parameters throughout gestation have been documented. For many indices related to cardiac function and maternal stress, it is known that changes in pregnancy tend to reach their maximal deviation from prepregnancy levels late in the second trimester or early in the third trimester. For several of these parameters, deviations from prepregnancy levels tend to diminish when gestation is approaching term. In labor dramatic changes ensue.

There has been intense interest in the endogenous opioid peptides during the past decade. β-Endorphin, a 31-amino acid peptide hormone, is thought to be involved in central nervous system mechanisms regulating pain tolerance[1]; it has also been shown to modulate luteinizing hormone and prolactin secretion by the human pituitary.[1] The main source of β-endorphin has been suggested to be the pituitary gland, which secretes adrenocorticotropic hormone and β-endorphin concomitantly since both are derived from a common 31 kilodalton precursor called proopiomelanocortin.[2] β-Endorphin has also been detected in other organs and fluids such as umbilical cord plasma,[3] endocrine pancreas,[4] amniotic fluid,[5] and placenta.[6]

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It is well appreciated that the opioid peptide is present in the newborn infant and in the fetal circulation.[1]

A few studies have made passing reference to possible maternal gestational age variation of β-endorphin levels or, more correct, β-endorphin immunoreactivity in normal pregnancies.[7-9] Unfortunately the number of samples in each category and the division merely into trimesters may not have been adequate to detect subtle differences.

Recent observations in our laboratory have suggested that measurement of plasma levels of β-endorphin immunoreactivity may underestimate the total circulating β-endorphin concentration by as much as 60%.[10] The elucidation of an erythrocyte pool of β-endorphin has helped to explain the highly variable results that have been noted by several groups in many clinical situations and indicates the problems associated with hemolyzed specimens. The purpose of the present investigation was to assess possible changes in β-endorphin immunoreactivity levels in both plasma and erythrocytes throughout the course of normal and complicated pregnancies.

Material and methods

β-Endorphin immunoreactivity levels in plasma and erythrocytes were measured in 118 pregnant women. These included 85 normal women at varying gestational ages, 11 gestational diabetic patients, nine patients with chronic hypertension, five with other medical problems, and eight patients in early labor. Specimens were obtained by venipuncture following informed consent. β-Endorphin levels were also measured retrospectively in 64 amniotic fluid specimens obtained at approximately 17 to 18 weeks of gestation for cytogenetic and/or biochemical analysis. The diagnoses of the patients were not known to the workers who performed the assay for β-endorphin immunoreactivity. Only when all of the results were obtained was the diagnosis disclosed to permit analysis of the data.

The method for determining β-endorphin levels in erythrocyte lysate has been described in detail.[10] Briefly, venous blood was placed into a polypropylene tube containing 3.8% sodium citrate as anticoagulant in a proportion of one part citrate to nine parts blood (v/v). This mixture was centrifuged at 1200 g and plasma separated, frozen, and lyophilized. The erythrocyte pellet was centrifuged and washed twice with two volumes of normal saline solution. The washed cells were then lysed by using deionized water in a ratio of 1 : 1. The lysate was frozen and lyophilized. The plasma and erythrocyte lysate samples thus prepared were reconstituted with equal volumes of radioimmunoassay buffer and assayed for β-endorphin immunoreactivity. The antibody to the New England Nuclear kit (North Billerica, Maine) was raised against human β-endorphin in rabbits and has a 50% cross-reactivity with β-lipotropin, whereas antibody obtained from Seragen (Boston, Mass.) did not have any cross-reactivity with β-lipotropin or enkephalins. Neither the Seragen nor New England Nuclear antibody crossreacted with human albumin, insulin, gamma globulin, leukocyte interferon, or hemoglobin. The limits of sensitivity are 5 pg per tube for the New England Nuclear kit and 6 pg per tube for the Seragen kit. The radioimmunoassay procedure for the Seragen kit was followed as recommended by the manufacturer; however, the procedure for the New England Nuclear kit was modified as follows: Reagent volumes were 200 μl; standards, 200 μl; tracer, 100 μl; antibody, 100 μl; and charcoal/buffer, 600 μl. Reported data are from the New England Nuclear kit. The Seragen kit was used to confirm the results obtained by using the New England Nuclear kit. Data obtained with the Seragen kit were within ± 1% of those obtained with the New England Nuclear kit and thus confirmatory.

To test the effects of gestational age in the five groups of patients on levels of plasma β-endorphin immunoreactivity, the following analyses were applied:

1. To obtain homogeneity of variance between the groups, the data were log transformed for the subsequent analysis.
2. Two-way analyses of variance were performed on the transformed data to test the main effects and interactions between them.
3. The hypothesis that each of the group means were equal was tested by use of Duncan's multiple range test.
4. (a) The graphs of the plasma β-endorphin immunoreactivity levels plotted against time suggested a relationship, perhaps a rise and fall or rise and plateau. (b) A straight-line fit in the data was attempted for 42 weeks of gestation but significant nonlinearity in the data was found. (c) The subsequent attempt was to fit a parabola by using an F test to see if there was a significantly better fit than the linear one. Finally, the data were split according to time factors into two linear segments at the point where the slope of the parabolic function was equal to zero. Confidence limits of 95% were calculated for each of these lines to see if they were different from zero and hence different from each other.
5. Approximate SEs* were calculated for all data.[1] SEMs are reported for all data; however, for erythro-

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*Approximate SEs are the difference between the upper and lower confidence limits divided by two times the appropriate t value for parameters mapped into the arithmetic scale (Table I).

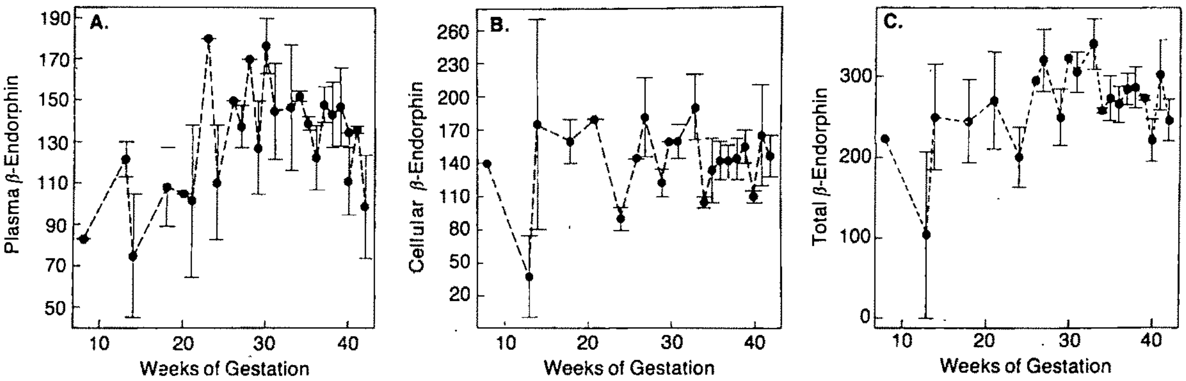

Fig. 1. β-Endorphin immunoreactivity curves over gestational age in normal patients. All values are reported as picograms per milliliter. *A*, Plasma β-endorphin immunoreactivity; *B*, cellular β-endorphin immunoreactivity; *C*, total (plasma and cellular).

Table I. Plasma and erythrocyte β-endorphin immunoreactivity levels in normal pregnancies

	β-Endorphin immunoreactivity (pg/ml)				
Determination	*10-14 wk*	*15-23 wk*	*24-32 wk*	*32-40 wk*	*41-42 wk*
Plasma					
N	5.0	11.0	15.0	48.0	6.0
Mean	95.2	113.1	145.9	139.7	111.8
SEM	±14.7	±13.1	±7.3	±5.2	±17.6
Upper confidence limit	136.0	142.3	161.6	150.3	157.0
Lower confidence limit	54.4	83.9	130.3	129.3	66.6
Erythrocyte					
N	4.0	6.0	13.0	41.0	6.0
Mean	122.7	136.7	154.5	134.0	148.1
SEM	42.3	19.6	13.9	6.8	15.7
Upper confidence limit	316.9	195.9	187.8	148.5	193.8
Lower confidence limit	47.5	95.4	127.1	121.0	113.9

The means and confidence limits for the erythrocyte β-IR levels were remapped from log computations because of the nongaussian distributions of the original data.

cyte β-endorphin immunoreactivity data in which the distribution is not normal, confidence limits are the more appropriate measure of variability.

6. Means and SEMs were calculated for each day of gestation wherever sufficient data were available ($n > 1$) for plasma, erythrocyte lysate, and total β-endorphin immunoreactivity (Fig. 1). When the sample size was 1, that value was plotted and no error bars were shown. Between 8 and 42 weeks of gestation, sample sizes varied from 2 to 6 with the exception of the thirty-seventh week when the sample size was 16.

Results

Plasma levels of β-endorphin immunoreactivity were found to increase significantly from early in the first trimester through approximately 32 weeks of gestation. From 32 weeks toward term and post dates, values were found to drift slightly downward although they were not statistically significant ($p > 0.05$) (Fig. 1, *A*, *B*, and *C*). The β-endorphin immunoreactivity level in erythrocytes was found to be stable throughout the course of pregnancy, and concentrations were higher at all gestational ages than they were in plasma (Table I).

Measurements of both plasma and cellular β-endorphin immunoreactivity levels were obtained in 11 diabetic patients. In a comparison of the overall mean levels with those of the normal patients, diabetic patients had a significantly lower level of plasma β-endorphin immunoreactivity ($p < 0.05$) and a statistically higher level of erythrocyte β-endorphin immunoreactivity ($p < 0.05$), although the total β-endorphin levels were indistinguishable (Table II). For patients with chronic hypertension and patients with other medical problems as well as those in early labor, there were no statistically significant differences in β-endorphin immunoreactivity levels when compared to levels in normal patients (Table II).

Assay of 64 midtrimester amniotic fluid samples for β-endorphin immunoreactivity levels revealed no differences on the basis of sex of the fetus (Table III).

Table II. Comparison of mean β-IR levels* in normal and complicated pregnancies

	β-Endorphin immunoreactivity (pg/ml)				
Determination	*Normal*	*Diabetic*	*Hypertensive*	*Other medical complications*	*Early labor*
Plasma					
N	85.0	11.0	9.0	5.0	8.0
Mean	125.9	90.0	97.0	98.1	93.0
SEM	5.8	7.7	25.4	31.4	21.3
Upper confidence limit	138.0	108.8	170.4	218.3	156.2
Lower confidence limit	114.9	74.4	55.3	44.1	55.4
Erythrocyte					
N	85.0	11.0	9.0	5.0	8.0
Mean	138.3	185.1	177.0	195.6	132.6
SEM	5.1	11.2	16.1	14.4	17.5
Upper confidence limit	148.8	211.8	217.2	239.7	180.4
Lower confidence limit	128.6	161.9	161.8	159.7	97.4

*Log remap

Table III. β-Endorphin immunoreactivity in midtrimester amniotic fluid samples

	Sex of fetus	
Determination	*Male*	*Female*
β-IR level (pg/ml)	84.1	85.2
±SD	±7.9	±8.4
N	30	34

Comment

Several groups have reported data on plasma β-endorphin immunoreactivity in maternal blood and also in amniotic fluid.[5, 7-9] Results from these and other studies have demonstrated that maternal β-endorphin is synthesized by the placenta,[11] and plasma levels rise markedly in labor. The β-endorphin immunoreactivity is detectable in amniotic fluid but concentrations decrease from the second to the third trimester (probably in association with dilution effects).

In response to significant stress, the fetus increases its release of β-endorphin, probably in a manner similar to that noted in adults.[12] Recent studies have established an inverse relationship between umbilical plasma β-endorphin immunoreactivity and umbilical artery Po$_2$ and pH, suggesting that fetal hypoxia may be related to β-endorphin release.[12] Shaaban et al[12] could not find any significant difference, in the absence of hypoxia, between umbilical plasma β-endorphin immunoreactivity levels of newborn infants with normal spontaneous vaginal delivery and those delivered by elective cesarean section. They further showed that neither the presence or absence of labor nor the route or mode of delivery affected β-endorphin concentrations in normal situations. However, in instances of fetal distress, neonatal plasma levels of β-endorphin immunoreactivity were significantly elevated when there was prolonged bradycardia, late or prolonged variable decelerations, or fetal acidosis. Furthermore, comparisons between umbilical venous and arterial β-endorphin concentrations show that venous β-endorphin concentrations were higher than arterial ones, suggesting a direct fetal input into whole-blood β-endorphin concentrations.

β-Endorphin levels in plasma have been reported to be undetectable to 1 ng/ml. Our observation of an erythrocyte pool of β-endorphin immunoreactivity may explain the wide variability noted in some other studies, possibly because of hemolysis of blood and/or release of the erythrocyte pool of β-endorphin immunoreactivity into plasma. The results of the study thus provide a better picture of the total level of β-endorphin immunoreactivity in circulation. Preliminary evidence suggests that release of erythrocyte-associated β-endorphin immunoreactivity may influence the plasma level of this neuropeptide in certain situations (unpublished data).

The statistically significant rise in β-endorphin immunoreactivity from the first trimester to the late second and early third trimesters is consistent with many other maternal physiologic adaptations to pregnancy. Physiologically, other indices of stress such as heart rate, left ventricular ejection fraction, and hematologic parameters such as red blood cell count and 2,3,-biphosphoglycerate concentrations all rise in this fashion and then decline toward term.[13, 14] It is unclear from our data whether the statistical decline from 32 through 42 weeks of gestation is real or merely represents a "plateau effect." The erythrocyte β-endorphin immunoreactivity shows a slight and statistically nonsignificant rise toward the period of midgestation. At all times, however, the concentration in the red blood cell is higher than it is in plasma.

In diabetic pregnancies the distribution of β-endorphin immunoreactivity seems to be somewhat altered from that in normal pregnancies. Although a role of β-endorphin in glucose metabolism has been suggested,[4] the reason for lower plasma and higher cellular fractions in diabetic patients is unclear. It is of interest that patients with hypertension and those with other medical problems did not show any statistically significant changes from normal, although the total number of such patients was not large. Similarly those patients in early labor did not manifest any differences, suggesting that the rise in the level of β-endorphin immunoreactivity may not be an early event in response to the stress of labor.

Many studies have attempted identification of fetal sex on the basis of midtrimester amniotic fluid hormone levels.[15] Our data suggest that β-endorphin immunoreactivity levels do not vary in relation to the sex of the fetus.

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Gestational age alters fetal breathing response to intravenous insulin and intravenous glucose administration

Paul M. Meis, M.D., James C. Rose, Ph.D., Melissa Swain, R.N., and Lewis H. Nelson, M.D.
Winston-Salem, North Carolina

An upward change in human maternal plasma glucose concentration is known to increase the percent of time spent in fetal breathing during the late third trimester of human pregnancy. We examined the fetal breathing effects of upward change in plasma glucose (after intravenous glucose administration) and downward change (after intravenous insulin administration) at two different times of day (8 AM and 4 PM) at both 24 and 36 weeks of gestation. No change in the percent of time spent in fetal breathing was seen after insulin infusion. Fetal breathing increased after glucose infusion at 36 weeks of gestation but not at 24 weeks. Responses did not differ between tests performed at 8 AM and 4 PM. (AM J OBSTET GYNECOL 1985;151:438-40.)

Key words: Fetal breathing, glucose, insulin, circadian variation

An increase in maternal plasma glucose concentration effected by either oral or intravenous glucose administration is consistently associated with an increase in fetal breathing activity in clinically healthy third-trimester human pregnancy.[1-3] Less information is available about the fetal breathing effects of lowering maternal glucose concentration. In addition, it has not been clear whether morning-afternoon differences exist in fetal breathing response to glucose alteration or whether alterations in maternal glucose concentrations influence fetal breathing prior to the third trimester. In an attempt to clarify these issues, we performed this study.

Material and methods

A group of 10 clinically healthy pregnant women were enrolled in this study after informed consent was obtained by means of a form approved by our institution's Clinical Research Practices Committee. Subjects with impaired glucose tolerance were excluded from the study. After an 8-hour fast these subjects received an intravenous bolus of either regular insulin (0.05 U per kilogram of body weight) or 25 gm of 50% glucose at 8 AM and 4 PM on separate days. Blood was drawn from a forearm vein by means of a heparin lock technique prior to and at 5, 10, 20, 40, and 60 minutes after the glucose infusion or prior to and at 15, 30, 45, and 60 minutes after the insulin infusion. These morning and afternoon tests were performed at either 24 weeks or 35 to 36 weeks of gestation. Because of logistic reasons, not all subjects were tested at both gestational times.

Fetal breathing activity was observed for 20 to 30 minutes prior to and for 60 minutes after the glucose or insulin infusion with the use of real-time ultrasound (ATL, ADR, Tempe, Arizona). An event marker was used to mark each fetal breath observed. Apnea was defined as the absence of breathing motion for 8 seconds. The presence of gross fetal body movements obscured detection of fetal breathing movements and was included in the amount of time counted as apnea. The time spent in gross fetal body movements was not recorded separately. The percent of time spent in fetal breathing was examined for control periods of 0 to 20, 20 to 40, and 40 to 60 minutes after glucose infusion and for control periods of 0 to 15, 15 to 30, 30 to 45, and 45 to 60 minutes after insulin infusion. Comparisons between results of morning and afternoon tests were examined by paired *t* test. Changes in plasma glucose and in percent of time spent in fetal breathing over the course of the tests were made for both insulin and glucose infusion studies at both 24 and 36 weeks of gestation by means of one-way analysis of variance.

Results

Comparison of results of 8 AM tests with the 4 PM tests at each gestational age studied showed no morning-evening difference in the following parameters: (1) rate of glucose clearance after glucose infusion, (2) maximal decrease in plasma glucose concentration after

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Table I. Response to glucose infusion

	Control periods	*Minutes after glucose infusion*			*n*	*Analysis of variance*	
		0-20	*20-40*	*40-60*		*F*	*p*
Mean plasma glucose concentration (mg/dl)							
Tests at 24 weeks	73.2 ± 5.0	186.3 ± 10.9	140.0 ± 9.5	106.4 ± 9.0	14	26.3	<0.005
Tests at 36 weeks	72.6 ± 2.7	192.8 ± 8.8	154.6 ± 9.1	121.3 ± 8.8	14	42.7	<0.005
Percent of time spent in fetal breathing							
Tests at 24 weeks	6.1 ± 2.0	6.6 ± 3.6	6.0 ± 1.5	7.1 ± 1.8	14	0.01	NS
Tests at 36 weeks	3.0 ± 1.0	8.6 ± 2.5	29.3 ± 4.2	41.5 ± 6.6	14	17.7	<0.005

Table II. Response to insulin infusion

	Control periods	*Minutes after insulin infusion*				*n*	*Analysis of variance*	
		0-15	*15-30*	*30-45*	*45-60*		*F*	*p*
Mean plasma glucose concentration (mg/dl)								
Tests at 24 wk	69.7 ± 3.6	60.1 ± 3.5	46.0 ± 3.7	44.6 ± 3.5	54.7 ± 3.5	14	8.4	<0.005
Tests at 36 wk	75.1 ± 5.3	69.9 ± 5.2	63.6 ± 5.1	63.6 ± 5.0	68.9 ± 4.6	11	0.9	NS
Percent of time spent in fetal breathing								
Tests at 24 wk	4.29 ± 0.9	8.5 ± 4.5	6.5 ± 1.6	2.4 ± 0.6	1.1 ± 0.3	14	1.7	NS
Tests at 36 wk	7.5 ± 2.7	5.8 ± 3.3	2.2 ± 1.0	3.0 ± 1.1	3.7 ± 2.0	11	0.8	NS

insulin infusion, and (3) fetal breathing response to maternal glucose or insulin infusion. Thus we find no evidence of a circadian variation in these maternal and fetal responses to alterations in plasma glucose in human pregnancy at 24 and 36 weeks of gestation.

Mean plasma glucose concentrations prior to and over 20-minute time intervals following intravenous glucose administration are shown in Table I. A similar rise and subsequent fall in glucose is seen with the tests at 24 and 36 weeks of gestation. Percent of time spent in fetal breathing during these tests is shown in Table I and Fig. 1. Although occasional fetal breathing movements are seen, no change in percent of time spent in fetal breathing occurs after glucose infusion at 24 weeks of gestation. In contrast, a sharp and significant increase in fetal breathing occurs approximately 20 minutes after glucose infusion at 36 weeks of gestation and reaches the highest observed values 40 to 60 minutes after the glucose infusion.

Table II shows mean plasma glucose concentrations and percent of time spent in fetal breathing prior to and over 15-minute time intervals following insulin infusion. A significant decrease in plasma glucose is seen after insulin infusion at 24 weeks' gestation, with lowest values found during the 30- to 45-minute time interval after insulin infusion. A decrease in plasma glucose cannot be demonstrated by analysis of variance at 36 weeks of gestation. No significant change in percent of time spent in fetal breathing occurs after insulin infusion at either gestational age.

Comment

The results of this study suggest that the fetal breathing response to maternal glucose intake is a developmental phenomenon dependent on gestational age. The response was clearly demonstrable at 36 weeks' gestation, but the percent of time spent in fetal breathing was not affected by glucose administration at 24 weeks. Previous studies[1-5] have examined this response only during the third trimester of human pregnancy.

Since percent of time spent in fetal breathing exhibits a circadian rhythm in both the sheep and human fetus,[6-8] we considered the possibility of a diurnal, or morning-evening, variation in the fetal breathing response to maternal glucose or insulin administration. In fact, no such morning-evening variation was found at either gestational age examined. This negative finding does not exclude the possibility of a circadian variation existing between other times of day not examined in this study.

Although the fetal breathing response to an increase in plasma glucose concentrations has been the subject of considerable study in human beings, little is known about the fetal breathing response to a decrease in plasma glucose concentrations from usual euglycemic levels. In these studies, the intravenous administration

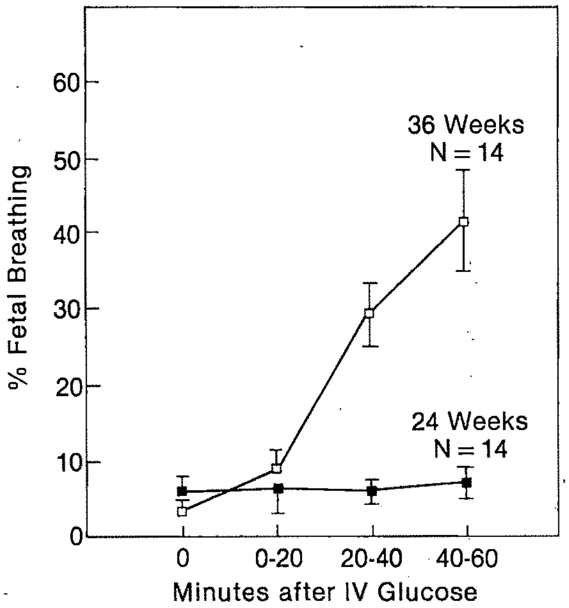

Fig. 1. Mean percent of time spent in fetal breathing prior to and during 20-minute time intervals after intravenous glucose bolus administration in subjects tested at 24 and 36 weeks of gestation.

of insulin significantly lowered maternal glucose concentrations at 24 weeks of gestation with no significant effect seen on percent of time spent in fetal breathing. The same dose of exogenous insulin had much less effect at 36 weeks of gestation, presumably because of the well-known progressive insulin resistance of pregnancy.[9] Again, no significant change occurred in percent of time spent in fetal breathing. The lack of effect on fetal breathing at 24 weeks' gestation is consistent with the observed lack of response to an increase in plasma glucose levels at this gestational age. It is possible that an alteration in fetal breathing after intravenous insulin administration may be detectable in third-trimester human pregnancies if a greater dose of insulin is used and/or if the insulin is administered during the fed state rather than the fasting state.

Since glucose-stimulated fetal breathing is a predictable phenomenon in healthy near term human pregnancy and since fetal asphyxia is known to be characterized by apnea,[6] several groups of investigators have attempted to use glucose-stimulated fetal breathing as a clinical measure of fetal well-being.[10, 11] If testing this fetal response is to be clinically useful, one must have knowledge of possible changes in this response at different times of the day and at different gestational ages. This study indicates no difference in this response between morning and afternoon tests but a marked difference in response between 24 and 36 weeks of gestational age. Further studies should be done to more closely examine the development of the fetal response to glucose infusion during human gestation.

We wish to thank John Patrick, M.D., for his suggestions and encouragement to perform this study.

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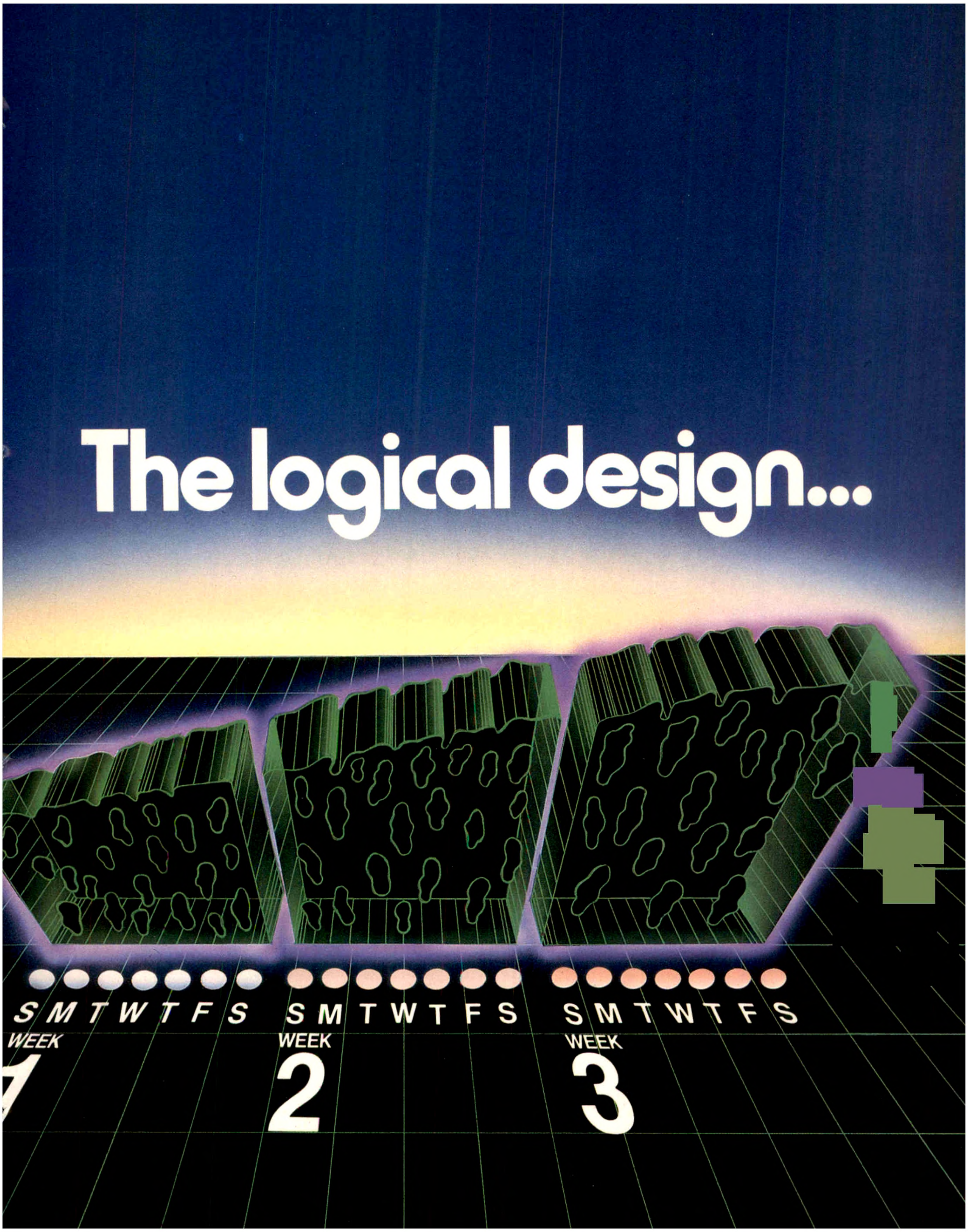
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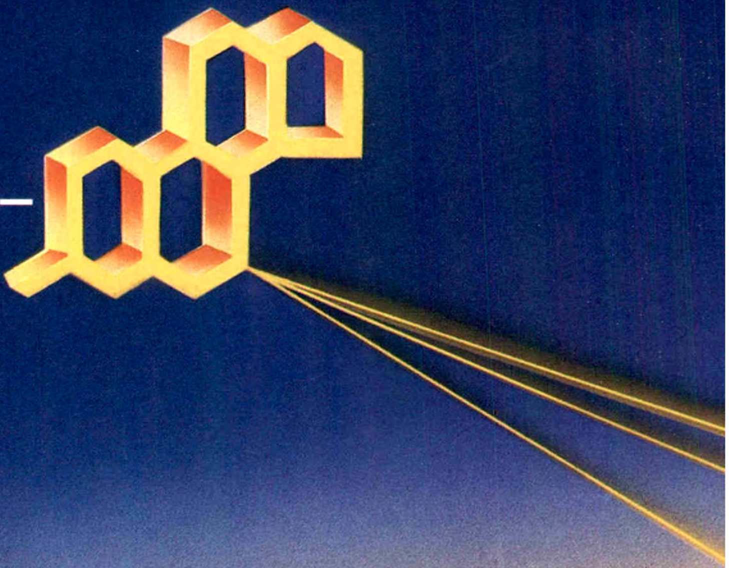

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.
DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.
CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1 Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4 Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use.
MYOCARDIAL INFARCTION: An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Precipitated opiate abstinence in utero

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Following direct infusion of morphine (0.6 to 80.0 mg/hr) to the fetal lamb for 2 to 6 hours, naloxone administration precipitated a fetal abstinence syndrome consisting of desynchronization of electrocortical activity; increased total body movements, neck tone, and eye movements; continuous rapid, deep breathing movements; immediate bradycardia associated with transient increases in systolic, diastolic, and pulse pressures; and meconium staining of the amniotic fluid. This syndrome resembles that observed in the opiate-abstinent human neonate. (AM J OBSTET GYNECOL 1985;151:441-4.)

Key words: Fetal withdrawal, morphine, physical dependence

Infants born to narcotic addicts are known to be at risk of undergoing an opiate-abstinence syndrome in the neonatal period. The most common manifestations of this syndrome include irritability, hyperactivity, increased eye movements, diarrhea, tachypnea, tremor, exaggerated reflexes, and occasionally generalized seizures. In addition, these infants exhibit abnormal sleep patterns, with an increase in wakefulness and rapid eye movement sleep, at the expense of quiet sleep.[1, 2, 3]

It is also known that detoxification of narcotic addicts during pregnancy results in fetal distress, as evidenced by meconium staining,[4, 5] increased amniotic fluid catecholamines, and occasional fetal death.[6] However, the complete nature of the opiate-abstinence syndrome has not been described for the human fetus.

Cohen et al.[7] reported changes in fetal hemodynamics and meconium staining following precipitated morphine withdrawal in pregnant ewes. However, they did not study the behavioral responses that might allow comparison with the abstinence syndrome described in the human neonate. Our objective was therefore to fully characterize the opiate-abstinence syndrome in the fetal lamb and compare it with that reported for the human neonate.

Material and methods

Animal preparation. Thirteen pregnant ewes and their fetuses (gestational ages ranging from 121 to 138 days at the time of study; term, approximately 145 days) were surgically instrumented for monitoring of fetal hemodynamics and behavioral activity at least 5 days prior to study. Details of the surgical procedure have been previously reported.[8, 9] Briefly, chronic indwelling catheters were placed in the fetal inferior vena cava for drug infusion and in the distal aorta for continuous monitoring of blood pressure and heart rate. For continuous monitoring of fetal behavioral activity, a pair of stainless steel screws were implanted extradurally over the parietal cortex to record electrocortical activity, a pair of stainless steel screws were implanted over the superior orbital ridge to record eye movements, and stainless steel electrodes were implanted in the dorsal neck muscles and the quadriceps femoris muscle to assess nuchal tone and body movements. In addition, fetal breathing movements were monitored via a pair of stainless steel electrodes implanted in the diaphragm and a catheter in the trachea.

Recording procedures. All recordings of fetal blood pressure, heart rate, tracheal pressure, electrocorticogram, electromyogram, and electrooculogram were carried out with the ewe standing or lying quietly in an experimental cart with free access to food and water. A companion sheep was kept in the same room to minimize restlessness.

All recordings were obtained with a Gould Model 2800 direct pen-writing analog recorder and appropriate amplifiers. The fetal arterial and tracheal catheters were connected to Statham Model P50 pressure transducers. Integrated fetal heart rate was obtained from the blood pressure recording by use of a Biotach coupler. The electrocorticogram, electromyogram, and electrooculogram were recorded via Universal amplifiers set at filter band widths of 0.3 to 30 Hz, 30 to 300 Hz, and 1 to 10 Hz, respectively.

Drug studies. Control records of fetal blood pressure, heart rate, and all neurobehavioral parameters were obtained for at least 2 hours prior to drug administration.

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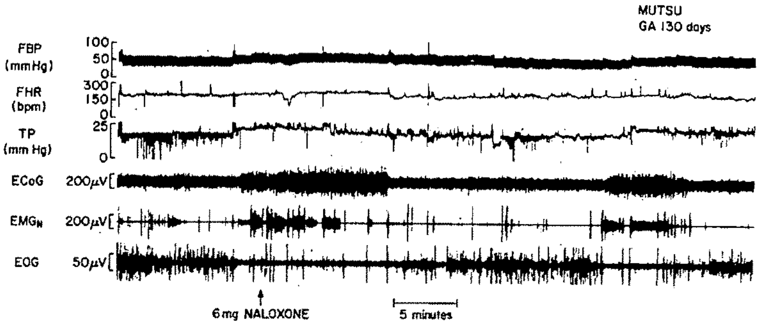

Fig. 1. The effects of naloxone on fetal blood pressure *(FBP)*, fetal heart rate *(FHR)*, tracheal pressure *(TP)*, electrocorticogram *(ECoG)*, nuchal electromyogram *(EMG$_N$)*, and electrooculogram *(EOG)* in an opiate-naive fetus.

In a preliminary study, naloxone was administered at doses of 0.75, 1.5, 3.0, 6.0, and 12.0 mg (intravenously) to opiate-naive fetuses, without any significant effect on the monitored physiologic variables. We chose a naloxone dose of 6.0 mg for use in all subsequent studies. This dose is comparable to that used in previous studies of specific opiate effects and precipitated opiate abstinence in rodents.[10, 11]

Morphine was infused into the fetus at a constant rate, ranging from 0.60 to 80.0 mg/hr for 2 to 6 hours. Naloxone was then administered to the fetus at the end of the morphine infusion. All hemodynamic and activity parameters were monitored continuously throughout the control period, during the morphine infusion, and for 2 hours following naloxone administration.

Data analysis. Heart rate and systolic and diastolic pressures were read from the traces at the end of morphine infusion and at the time of maximal change following naloxone administration. The mean differences (±SE) were reported and their significance determined by use of the nonparametric sign test.[12]

Results

A total of 20 withdrawal experiments were performed on 13 animals. On the day of the drug study, maternal and fetal blood gases and pH were within normal limits. All control recordings showed cyclic variation in electrocortical activity, eye movements, total body movements, and breathing movements, as previously reported.[8, 9, 13]

The administration of morphine resulted in two distinct response clusters[14] (Umans and Szeto, unpublished observations) as a function of dose. Low doses (up to 2.5 mg/hr) resulted in desynchronization of the electrocorticogram; increased nuchal tone, body and eye movements; continuous fetal breathing movements; and increased heart rate and heart rate variability[9] (on the left in Fig. 2). Higher doses (through 80.0 mg/hr) resulted in a return of synchronized electrocorticogram activity, accompanied by decreases in body, eye, and breathing movements and heart rate variability (on the left in Fig. 3).

The administration of naloxone to opiate-naive fetuses did not result in any alteration in the physiologic parameters that were monitored or in their pattern of cyclic variation (Fig. 1).

Following low-dose morphine infusion, naloxone administration resulted in further desynchronization of the electrocorticogram, an immediate bradycardia, an increase in both the rate and depth of breathing movements, and an increase in total body movements (Fig. 2). In some instances it also resulted in a subsequent reversal of the acute effects of morphine, with a return to cyclic variation in electrocorticogram activity, eye movements, nuchal tone, and electrocorticogram-associated breathing movements.

Following high-dose morphine infusion, administration of naloxone provoked a qualitatively similar but more intense abstinence syndrome (Fig. 3).

Following morphine infusion, naloxone administration resulted in increased systolic ($\Delta = 18 \pm 3$ mm Hg, $p < 0.01$), diastolic ($\Delta = 7 \pm 2$ mm Hg, $p < 0.01$), and pulse ($\Delta = 11 \pm 2$ mm Hg, $p < 0.01$) pressures, with a decrease in heart rate ($\Delta = -74 \pm 8$ bpm, $p < 0.01$). These changes did not vary as a function of the dose or duration of morphine infusion.

Further, following morphine infusion, naloxone administration precipitated severe meconium staining of the amniotic fluid. In two sets of twins delivered by cesarean section at term, meconium staining was only observed in the drug-exposed twin.

In fetuses that were subjected to an episode of precipitated abstinence following morphine infusions of $\geq$25.0 mg/hr, a second episode of abstinence could be precipitated by naloxone administration 24 hours following the initial withdrawal. Immediately prior to administration of the second dose of naloxone, all fetuses exhibited normal control traces without evidence of acute opiate effect. The second episode of abstinence was in each case similar to but less intense than the first.

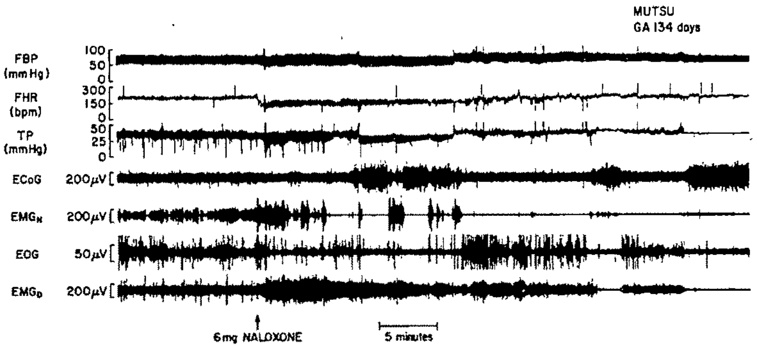

Fig. 2. The effects of naloxone on fetal hemodynamic and behavioral activity parameters when administered subsequent to a 6-hour infusion of morphine at 2.5 mg/hr. *EMG*$_d$, Diaphragmatic electromyogram.

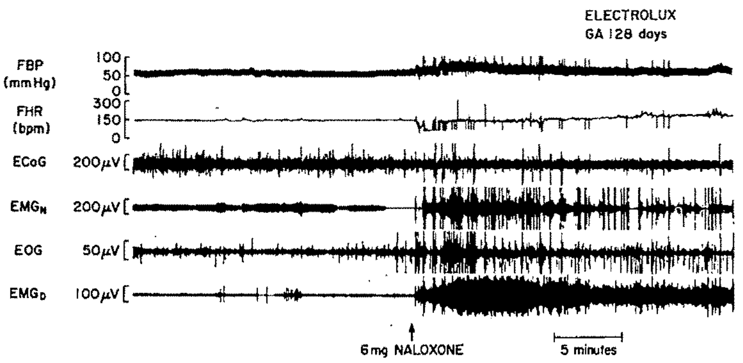

Fig. 3. The effects of naloxone on fetal hemodynamic and behavioral activity parameters when administered subsequent to a 6-hour infusion of morphine at 25.0 mg/hr.

Comment

It is generally assumed that neonatal morbidity and developmental abnormalities following intrauterine narcotic exposure result from repeated episodes of fetal withdrawal.[15] We have previously demonstrated direct effects of opiate administration on fetal neurobehavioral function which may in themselves account for the effects observed in the neonate.[8, 9] We now report data which suggest that both direct opiate pharmacodynamic effects and fetal withdrawal may contribute significantly to neonatal outcome following intrauterine narcotic exposure.

Using the chronically instrumented fetal lamb preparation, we have characterized a syndrome of naloxone-precipitated opiate abstinence following short-term infusion of morphine into the fetus. The syndrome consists of desynchronization of electrocortical activity; increased total body movements, neck tone, and eye movements; continuous, rapid deep breathing movements; an immediate bradycardia associated with transient increases in systolic, diastolic, and pulse pressures; and meconium staining of the amniotic fluid. This syndrome resembles the one observed in the human neonate in that the human exhibits increased rapid eye movement sleep and wakefulness at the expense of quiet sleep, increased body and eye movements, diarrhea, tachypnea, and hyperpnea.[1, 2, 3] The syndromes differ, however, in that we observed no cortical seizure activity in the fetal lamb. There are no human data available describing the cardiovascular manifestations of neonatal withdrawal.

Based on the present observations, we agree with Zuspan et al.[6] in suggesting that intrauterine opiate withdrawal presents a severe physiologic stress to the developing fetus. Our data, which show that a significant abstinence syndrome may be precipitated following short (i.e., 2 hours), low-dose fetal exposure to morphine, further suggest that the fetus may undergo a cycle of acute physical dependence and withdrawal following the administration of opiates in normal clinical practice.

We are most grateful for the excellent technical assistance contributed by Hilary R. Umans.

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The effect of prostacyclin on angiotensin II–induced placental vasoconstriction

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Significant alterations in vascular responsiveness to angiotensin II have been documented during pregnancy. We have observed that prostacyclin, a potent vasodilating prostaglandin, does not dilate the ovine placental vasculature. However, we thought that it might modulate the placental vasoconstriction produced by angiotensin II. Regional blood flows and resistances were measured by the radioactive microsphere technique in six near-term sheep. Blood flows were measured in the control condition and 15 minutes after beginning an infusion of angiotensin II at 5 μg/min (T_1). Additional measurements were made 15 minutes after the addition of 50 μg/min of prostacyclin to the angiotensin II infusate (T_2) and 15 minutes after withdrawing prostacyclin from the angiotensin II infusion (T_3). Mean arterial pressure rose in response to angiotensin II and decreased significantly with prostacyclin administration. The renal and uterine nonplacental vascular beds showed the expected vasoconstriction in response to angiotensin II, which was then reversed to control levels by prostacyclin infusion. Unexpectedly, prostacyclin did not reverse the angiotensin II vasoconstriction in the placenta but further increased resistance ($p < 0.03$). Placental resistance changed from 0.33 ± 0.04 peripheral resistance units in the control condition to 0.42 ± 0.06 peripheral resistance units for T_1 ($p < 0.03$), and prostacyclin infusion further increased placental resistance to 0.63 ± 0.10 peripheral resistance units for T_2 ($p < 0.03$). We conclude that the placental vascular response to prostacyclin is different from that of other organs and that prostacyclin does not dilate, but further constricts the placenta in the near-term sheep with angiotensin II–induced systemic vasoconstriction. (Am J Obstet Gynecol 1985;151:444-9.)

Key words: Prostacyclin, placenta, angiotensin II

Prostacyclin is a potent vasodilating prostaglandin[1] formed by the cyclooxygenase metabolism of arachidonic acid. It is synthesized by many tissues including the uterus[2] and placenta[3] and is felt by some investigators to have a role in the normal vascular adaptation to pregnancy.[4]

Prostacyclin has been shown to dilate the uterine vasculature in the nonpregnant ewe[5] and the uterine placental circulation in the pregnant ewe when measured

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by electromagnetic flow probe.[6] However, blood flow studies with the use of radioactive microspheres have shown an anomalous maternal placental vasoconstrictive response to the systemic administration of prostacyclin, while the noncotyledonary uterine vascular bed shows the expected dilation.[7] Blood flow measurements made after local administration of prostacyclin via a retrograde uterine artery catheter, without systemic hypotension, confirm the observation that prostacyclin does not dilate the maternal placental circulation.[8]

The observation that prostacyclin does not appear to dilate the placental vasculature in its normal, maximally dilated state, led us to postulate a vasodilating role for prostacyclin in the vasoconstricted state. This study was designed to test the hypothesis that prostacyclin would modulate angiotensin II–induced placental vasoconstriction.

Material and methods

Six pregnant ewes ranging from 120 to 125 days of gestation were used in this experiment. The animals were anesthetized with ketamine, 10 mg/kg, and atropine, 0.6 mg intramuscularly, supplemented by ketamine intravenous infusion at 10 mg/min. Polyvinyl catheters were placed into a branch of the maternal femoral artery bilaterally and advanced for 20 cm to lie in the distal abdominal aorta. A stiff polyethylene catheter surrounding a standard polyvinyl catheter was placed into the carotid artery and advanced into the left ventricle, where placement was confirmed by characteristic pressure pattern. The maternal jugular vein was also catheterized. The abdomen was then opened through a midline incision and a fetal hindlimb brought out through an incision in the uterine wall. The ventral surface was anesthetized with 1% Xylocaine, and polyvinyl catheters were placed in the fetal hindlimb artery and vein and advanced for 20 cm. All catheters were filled with 100 U/ml of heparin; chloramphenicol, 250 mg, was given via the fetal vein, and 600,000 U of aqueous penicillin G was instilled into the amniotic cavity while an additional 400,000 U was given intramuscularly to the ewe. Fetal hindlimb, uterine, and abdominal wall incisions were closed, and the catheters were tunneled subcutaneously and secured in a pouch on the maternal flank. The jugular vein and carotid artery catheters were secured to the neck with an elastic bandage. The animals were allowed to recover from surgery for 4 days before experimentation. Each animal was returned to the laboratory and stabilized for 12 to 24 hours before experimentation, which was performed with the ewe standing quietly in a stanchion.

Maternal mean arterial pressure was continuously recorded with a R411 Beckman physiologic recorder via a Statham P23Db transducer attached to a femoral artery catheter. Maternal placental blood flow was expressed as ml · min^{-1} · kg^{-1} of fetal weight. Renal and uterine nonplacental vascular resistance was defined as mean arterial pressure in mm Hg divided by total organ blood flow in ml · min^{-1} and expressed as peripheral resistance units. Placental vascular resistance was defined as mean arterial pressure in mm Hg divided by placental blood flow in ml · min^{-1} · kg^{-1} of fetal weight and expressed as peripheral resistance units (kg^{-1}).

Regional blood flows were measured by the radioactive microsphere method. In this experiment, microspheres that were 15 μm in diameter and labeled with ^{125}I, ^{57}Co, ^{85}Sr, and ^{46}Sc were used. Each injection contained approximately 2×10^6 microspheres suspended in 0.4 ml of 10% dextran. We injected the microspheres into the maternal left ventricle while simultaneously withdrawing an integrated arterial sample from a maternal femoral artery catheter at 4.12 ml/min for 1.5 minutes starting from the time of microsphere injection.

Experimental design. Regional blood flows were measured in the control condition. An infusion of Val 5 angiotensin II at a dose of 5 μg/min in normal saline solution was then begun via the jugular vein catheter. Regional blood flows were measured after 15 minutes of angiotensin II infusion (T_1), and the infusion continued at the same dosage throughout the remainder of the experiment. Prostacyclin was then added to the angiotensin infusion at a dosage of 50 μg/min, and regional blood flows were measured for the third time 15 minutes later (T_2). Prostacyclin was removed from the angiotensin II infusion, and the final blood flow measurement (T_3) was made 15 minutes later.

The prostacyclin (Upjohn Company) was stored in a freezer and then diluted with normal saline solution immediately prior to infusion.

Radioactive assay. The animals were sacrificed at the end of the experiments and the kidneys and uterus removed for assay. The placental cotyledons were dissected away from the endomyometrial uterine tissue, and the fetal membranes were discarded. Cotyledonary tissue was cut into slices, weighed, and placed in glass counting vials. The kidneys and uterine nonplacental tissues were homogenized in a Waring blender with known quantities of water, and 10 aliquots containing 3 ml of the homogenate were placed in counting vials. The height of the samples in the vials never exceeded 1 cm. Vials containing a known number of microspheres of each isotope embedded in wax 0.5 cm from the bottom of the vial were used as standards.

Radioactivity was assayed in a Nuclear Chicago 1185 three-channel automatic gamma counter connected to a Univac 1110 computer. Appropriate corrections were made for the spillover of each isotope into the other channels. The number of microspheres in each organ and the blood flow to that organ were calculated by the equations previously described,[9] and run on computer

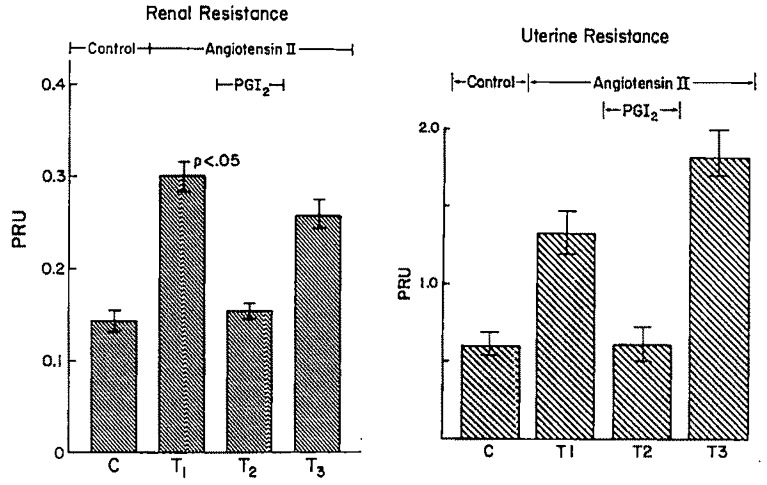

Fig. 1. Response of renal and nonplacental uterine resistance to angiotensin II and prostacyclin (*PGI_2*). Measurements were made in the control condition *(C)*, after angiotensin II infusion *(T1)*, after addition of prostacyclin to the angiotensin II infusion *(T2)*, and finally after removing prostacyclin from the angiotensin II infusion *(T3)*. The decrease in renal and nonplacental uterine resistance from *T1* to *T2* is statistically significant ($p < 0.05$). *PRU*, Peripheral resistance units.

programs developed by our laboratory. All organ samples contained more than 400 microspheres, which satisfies the criteria of Buckberg et al.[10] for the accuracy of blood flow measurement by this method.

The Wilcoxon signed-rank test was used to evaluate the difference between the means of the control group and T_1, T_1 and T_2, and T_2 and T_3. The paired *t* test was additionally applied to evaluate the difference between T_1 and T_2, since this is the critical comparison in our study. Because resistance values represent calculated ratios and because the ratios of two normally distributed populations are not normally distributed, logarithmic transformation was performed on all resistance values. A probability of $p < 0.05$ was chosen to represent statistical significance. Data are expressed as mean ± standard error of the mean.

Results

Maternal and fetal arterial blood gases were drawn before each experiment to determine well-being. The mean maternal pH was 7.51 ± 0.01, while fetal pH was 7.38 ± 0.02. The mean fetal weight in this series was 3.63 ± 0.42 kg.

The effect of angiotensin II administration at 5 μg/min on all organs studied was predictable and consistent with that of other investigators[11, 12] and our previous experiments.[13] Angiotensin II raised the mean arterial pressure from 84 ± 6 to 103 ± 6 mm Hg ($p < 0.05$) while also increasing vascular resistance in the kidney (0.14 ± 0.02 to 0.30 ± 0.04 peripheral resistance units, $p < 0.05$) and in the nonplacental uterine bed (0.60 ± 0.09 to 1.35 ± 0.31 peripheral resistance units, $p < 0.05$). The placental vasculature was relatively refractory to angiotensin II infusion, with resistance increasing significantly from 0.33 ± 0.04 to 0.42 ± 0.06 peripheral resistance units ($p < 0.03$) but placental blood flow remaining unchanged (272 ± 33 to 264 ± 33 $ml \cdot min^{-1} \cdot kg^{-1}$ of fetal weight, $p < 0.3$). Placental angiotensin II refractoriness during pregnancy has also been observed by Rosenfeld and Naden.[12]

The addition of prostacyclin to the angiotensin II infusion resulted in the expected responses from most organ systems. Mean arterial pressure was reduced from 103 ± 6 to 66 ± 4 mm Hg ($p < 0.05$). Renal resistance decreased from 0.30 ± 0.04 to 0.15 ± 0.03 peripheral resistance units ($p < 0.05$) and the uterine nonplacental bed reacted with similar vasodilation, decreasing its resistance from 1.35 ± 0.31 to 0.62 ± 0.10 peripheral resistance units ($p < 0.05$). Both renal and uterine nonplacental vasculatures responded to prostacyclin by decreasing their resistance significantly ($p < 0.05$) towards the normal control levels. This observation is graphically represented in Fig. 1.

Unexpectedly, the placenta responded to prostacyclin in a manner different from all other organs. Placental resistance, which was relatively refractory although significantly increased ($p < 0.03$) in response to angiotensin II alone, showed further vasoconstriction (0.42 ± 0.06 to 0.62 ± 0.10 peripheral resistance units, $p < 0.03$) when prostacyclin was added to the angiotensin II infusion. Placental blood flow was similarly affected, being refractory to angiotensin II (272 ± 33 to 264 ± 33 $ml \cdot min^{-1} \cdot kg^{-1}$ of fetal weight and decreasing significantly to 120 ± 21 $ml \cdot min^{-1} \cdot kg^{-1}$ of fetal weight ($p < 0.004$) in response to the ad-

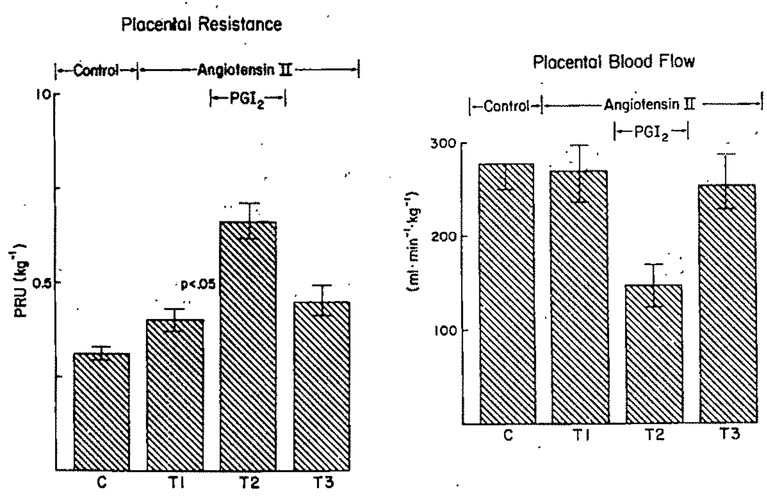

Fig. 2. Placental vascular responses to angiotensin II and prostacyclin *(PGI$_2$)*. Measurements were made in the control condition *(C)*, after angiotensin II infusion *(T1)*, after addition of prostacyclin to the angiotensin II infusion *(T2)*, and finally after removing prostacyclin from the angiotensin II infusioin *(T3)*. The decrease in placental blood flow from *T1* to *T2* is statistically significant ($p < 0.004$).

Table I. Placental vascular responses to systemic infusion of angiotensin II (T_1) and after addition of prostacyclin to the angiotensin II infusion (T_2)

Animal No.	*Placental blood flow (ml · min⁻¹ · kg fetal weight⁻¹)* T_1	T_2	*Placental vascular resistance (peripheral resistance units, kg⁻¹)* T_1	T_2
1	180.4	76.4	0.53	0.76
2	193.4	102.2	0.65	0.83
3	292.8	129.8	0.33	0.46
4	208.1	147.4	0.41	0.49
5	352.2	201.9	0.28	0.32
6	357.9	63.6	0.33	0.91
Mean ± SEM	264.3 ± 33	120.2 ± 21	0.42 ± 0.06	0.63 ± 0.1
p	<0.004		<0.03	

dition of prostacyclin. These placental vascular responses are graphically depicted in Fig. 2 and numerically represented in Table I.

Withdrawal of prostacyclin from the angiotensin II infusion uniformly resulted in return of all organ resistances and placental blood flow towards T_1 values (Figs. 1 and 2).

Comment

Normal pregnancy is associated with decreased vascular reactivity to the pressor effects of angiotensin II in both humans[14] and sheep.[11] When distinct organ vascular responses are studied, the placental vascular bed appears to be the most protected from angiotensin II vasoconstriction.[12]

Women who develop preeclampsia, a common vasoconstrictive disorder of pregnancy classically accompanied by hypertension, edema, and proteinuria, lose their refractoriness to angiotensin II and become sensitive to its pressor effects even before clinical manifestations of the disease are apparent.[14] This disorder has also been associated with a significant decrease in placental perfusion as evidenced by the decreased clearance rate of dehydroisoandrosterone sulfate.[15] Chronically hypertensive gravid women who are destined to become preeclamptic also show the same sensitivity to angiotensin II.[16] It is clear from these observations that vascular reactivity to angiotensin II and placental function are markedly altered by the pathophysiology of certain vasoconstrictive disorders of pregnancy.

Prostacyclin, known to have potent vasodilating effects on vascular tissue,[1] is synthesized in significant quantities in the pregnant myometrium and placenta as well as in fetal umbilical and maternal placental vascular endothelium.[3, 17] Prostacyclin production in umbilical and placental vascular tissues is significantly decreased in women with severe preeclampsia in comparison to that of normal pregnant women.[17] Behr et al.[3] have shown that placental nonvascular tissue from preeclamptic women has a marked decrease in prostacyclin production when compared with the same tissue from normal pregnant women.

It has been postulated that the relative lack of prostacyclin in preeclamptic pregnancies may contribute to the generalized vasoconstriction and multiorgan dysfunction associated with this disease.[17] Because the placenta and its vascular endothelium are major sites for prostacyclin production, the placental vasoconstriction of preeclampsia is thought to be directly related to the documented deficiency of this vasodilator.[3, 17] This led several investigators to speculate that the vasoconstriction associated with severe preeclampsia might be reversed by administration of exogenous prostacyclin.[17, 18]

There are three case reports in the literature to date in which prostacyclin infusions have been used to treat women with severe preeclampsia.[18, 19] In all three cases, maternal hypertension responded nicely to the infusions which lasted from 3 to 12 days. All patients were between 25 and 27 weeks of gestation at the initiation of therapy. Fetal death occurred unexpectedly and without warning in two of the three patients in spite of improvement in maternal condition. The third fetus had three documented episodes of bradycardia after 3 days of maternal prostacyclin therapy and was delivered at 27 weeks' gestation by cesarean section because of fetal distress.

The present study was undertaken in an attempt to show that prostacyclin would prove to be a placental vasodilator in the face of systemic vasoconstriction caused by angiotensin II. Our data convince us that not only was this hypothesis incorrect, but perhaps more importantly, that administration of prostacyclin to the systemically vasoconstricted sheep results in a significant increase in placental vascular resistance and decrease in placental blood flow. This anomalous response of the placental vascular bed in our study leads us to question whether this observation bears any physiologic similarity to the clinical cases reviewed above. Given our data, the possibility exists that administration of exogenous prostacyclin to the preeclamptic patient may cause sufficient increased placental vasoconstriction in an already compromised vascular bed, as to be deleterious to fetal health.

We have considered several possible sources of error in the interpretation of our results. We have rejected the possibility that the prostacyclin used was inactive, because we saw significant vasodilation in all other vascular beds and because the drug was stored frozen and prepared immediately prior to use.

Endogenous catecholamine release may have affected our results, and since we did not measure catecholamine levels in these animals, we cannot reject this possibility. It is, however, unlikely that catecholamine release would have affected the placental bed to the exclusion of the myometrium, kidneys, and the systemic vasculature. In addition, our laboratory has previously shown that prostacyclin does not dilate the maternal placental circulation in the face of norepinephrine-induced vasoconstriction.[13]

We are left to conclude that prostacyclin does not dilate the maternal placental vascular bed during angiotensin II–induced vasoconstriction. Furthermore, prostacyclin significantly worsens the placental vascular responses to angiotensin II, increasing resistance by 48% ($p < 0.05$) and decreasing placental blood flow by 56% ($p < 0.004$). It follows from these data that the maternal placental vascular response to prostacyclin in the constricted state is both anomalous and unique.

We wish to thank Dr. John Pike of the Upjohn Company for supplying the prostacyclin with which this research was conducted.

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A comparison of sheep and human fetal oxygen delivery systems with use of a mathematical model

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Human fetal cardiac output measured with ultrasound is only about 60% of that found in the sheep. We modified a previously developed mathematical model of the fetal circulation and oxygen delivery in sheep for the human in order to study several differences. The model predicts that a human fetus can maintain its oxygen delivery with a relatively low cardiac output because of its relatively high fetal hemoglobin concentration, as compared with that of the sheep fetus. Thus an inverse relationship between fetal hemoglobin concentration and fetal cardiac output is suggested. This relationship may be mediated by the influence of red blood cell concentration on blood viscosity. Furthermore, it indicates that fetal anemia should be detectable by ultrasound measurements of increased cardiac output and/or umbilical blood flow. Dynamic responses of the model suggest that the mechanism of late and variable decelerations in the fetal heart rate pattern is mediated via a fall in arterial oxygen tension. (AM J OBSTET GYNECOL 1985;151:449-55.)

Key words: Ultrasound measurement of fetal cardiac output, blood viscosity, fetal anemia

There is a striking difference between values of cardiac output and umbilical blood flow measured in the human and lamb fetus. Cardiac output and umbilical blood flow in the fetal lamb have been measured with electromagnetic flow probes, and dye, thermodilution, and microsphere techniques.[1-3] In the human fetus, measurements have been made with ultrasound and Doppler techniques.[4-6] Reported values for cardiac output and umbilical flow in humans are about 60% of those reported for the healthy, chronically catheterized fetal lamb. One may conclude that these differences result from different techniques and favor the more numerous measurements and varied techniques reported for the fetal lamb over the limited human results with noninvasive methods. However, the flow values reported in humans with Doppler techniques are consistent with cardiac output values computed from changes in ventricular dimensions measured with two-dimensional real-time ultrasound,[4] findings which support a real cross-species difference.

It is true that in acute experiments with exteriorized fetal lambs, values of cardiac output as low as those measured in the human have been reported[1]; however, results of chronic experiments indicate that such values are incompatible with normal oxygen delivery to the fetal lamb. In addition, estimates of fetal oxygen consumption indicate that this value is similar in humans and sheep.[7]

To study more thoroughly these apparently irreconcilable differences between the human and the sheep fetus, we used a previously described mathematical model of the fetal circulation and oxygen delivery to explore how several known differences between these

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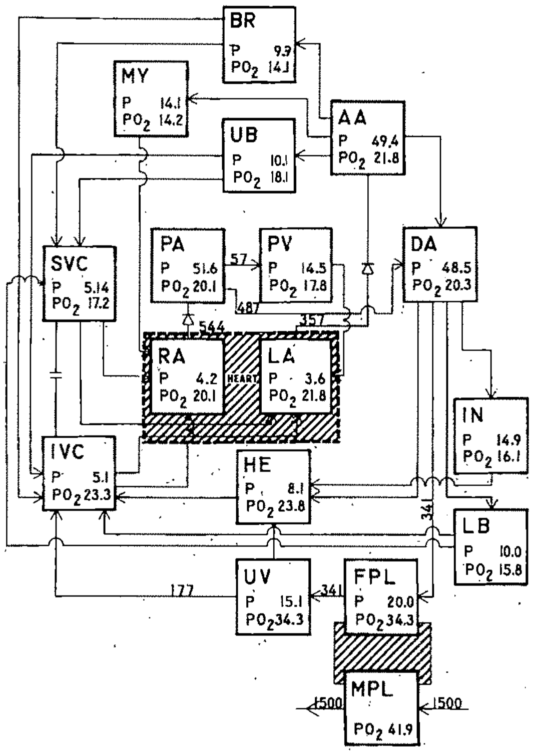

Fig. 1. Schematic of the overall structure of the model. *AA*, Ascending aorta; *BR*, brain; *DA*, descending aorta; *FPL*, fetal side placenta; *HE*, liver; *IN*, intestines; *IVC*, inferior vena cava; *LA*, left atrium; *LB*, lower body; *MPL*, maternal side placenta; *MY*, myocardium; *PA*, pulmonary artery; *PV*, pulmonary bed; *RA*, right atrium; *SVC*, superior vena cava; *UB*, upper body; *UV*, umbilical vein(s). Steady-state values of the model scaled to a 3 kg human fetus for pressures and oxygen tensions. *P*, Blood pressure in millimeters of mercury; Po_2, oxygen tension in millimeters of mercury. Flows are in milliliters per minute.

species change the predictions of the model. Table I lists the differences we examined between sheep and humans. The model allows us to study theoretically the effects of a decrease in cardiac output, an increase in hemoglobin concentration, and a change in blood viscosity in fetal sheep. Both the effects on steady state and the dynamic responses to transient decreases in maternal or fetal placental flow were explored. The results suggest a theoretical basis to explain how results in experimental animals can differ from those in humans.

Methods

We have previously described in detail the mathematical model of the fetal circulation and oxygen delivery.[8] Summarizing briefly, the fetal circulation was divided into 16 compliant compartments as shown in Fig. 1. Blood pressure in each compartment was determined by its stressed volume and compliance. Lines connecting the compartments indicate possible flow, which, in general, is linearly related to the pressure difference between the two compartments. Resistance to flow is located between compartments, that is, each compartment representing an organ has an inflow and outflow resistance. Cardiac output was derived from cardiac function curves for both ventricles. The maternal placental compartment was modeled as a pool with constant input and output of blood. Placental oxygen exchange was linearly related to the oxygen tension difference between the maternal and fetal placental exchange vessels. Each compartment has an oxygen content with its oxygen inflow being the product of the blood flow and the oxygen concentration of the inflowing blood. Oxygen outflow is modeled in a similar fashion. In compartments representing an organ system, the oxygen consumption is modeled by extracting oxygen at a constant rate within the compartment. Baroreceptors and chemoreceptor responses are not included.

Table I. Explored differences in parameter values

Parameter	*Sheep value*	*Human value*
Fetal cardiac output ($ml \cdot min^{-1} \cdot kg^{-1}$)	444	279
Fetal hemoglobin level ($gm : 100 ml^{-1}$)	11	17
Constants for fetal hemoglobin oxygen dissociation curve		
k_1	1.32	1.32
k_2	0.32	0.39
Relative viscosity of fetal blood (water = 1)	3.3	5.2
Constants for maternal hemoglobin oxygen dissociation curve		
k_1	1.62	1.44
k_2	0.22	0.39
Maternal hemoglobin level ($gm \cdot 100 ml^{-1}$)	10	12
Maternal oxygen saturation (%)	85	90
Overall placental oxygen diffusing capacity ($ml \cdot min^{-1} \cdot mm Hg^{-1} \cdot kg^{-1}$)	0.83	1.66

The model was scaled for a 3 kg fetal lamb. As far as possible, values for parameters were derived from chronic sheep experiments. We refer to our detailed description[8] for the normal steady-state values for blood volumes, pressures, oxygen tensions, and flows used in the model.

Using the model, we explored how several steady-state values would change if selected parameters were scaled to human values. To scale the cardiac output to a human value, we decreased it but maintained the normal sheep distribution of blood flow, pressures, and volumes and oxygen consumption. We produced these

Table II. Values for organ blood flow and oxygen tension for the sheep model with changed cardiac output and hemoglobin concentration

	Cardiac output: 444 $ml \cdot min^{-1} \cdot kg^{-1}$ Hemoglobin: 11 $gm \cdot 100\ ml^{-1}$		*Cardiac output: 279 $ml \cdot min^{-1} \cdot kg^{-1}$ Hemoglobin: 11 $gm \cdot 100\ ml^{-1}$*		*Cardiac output: 279 $ml \cdot min^{-1} \cdot kg^{-1}$ Hemoglobin: 17 $gm \cdot 100\ ml^{-1}$*	
Organ	*Flow ($ml \cdot min^{-1}$)*	*Po_2 (mm Hg)*	*Flow ($ml \cdot min^{-1}$)*	*Po_2 (mm Hg)*	*Flow ($ml \cdot min^{-1}$)*	*Po_2 (mm Hg)*
Brain	22	15.6	14	0	14	15.2
Intestines	19	17.4	12	10.5	12	17.0
Liver	10*	23.8	6*	20.5	6*	23.6
Lower body	90	17.1	56	9.7	56	16.7
Lungs	30	18.8	19	13.3	19	18.5
Myocardium	20	15.7	12	0	12	15.3
Placenta	180†	32.4†	112†	32.5†	112†	32.1†
Upper body	105	19.0	66	13.7	66	18.7

*Arterial blood supply.
†Fetal side.

changes in the system by multiplying all resistances by a factor, while simultaneously dividing the cardiac function values by the same factor. For instance, a 50% decrease in cardiac output resulted from an increase of all resistances to 200% of their values and a 50% decrease of the cardiac function values.

We also examined the effects of differences between human and sheep fetal hemoglobin concentrations, oxygen dissociation curves, maternal hemoglobin concentrations, and maternal oxygen tensions by changing the appropriate model parameters. Total oxygen consumption and blood volume do not differ substantially between species. Because of the lack of data on humans, we used sheep values for the distribution of blood volumes, pressures, and flows and oxygen consumption. As a result, the theoretical model is scaled for a lamb with the central circulation adjusted to human values. Some of the possible errors resulting from these assumptions are discussed later.

We explored effects of different hemoglobin concentrations on blood viscosity and thus cardiac output. Our computations assumed that a hemoglobin value of 3.4 gm/100 ml corresponds to a hematocrit of 10%, which allowed us to relate viscosity directly to the hemoglobin concentration.[9, 10] We also studied the dynamic responses in both species to transient decreases in maternal and fetal placental blood flow.

Results

Cardiac output, hemoglobin, and oxygen delivery. The combined cardiac output in the fetus is the sum of both ventricular outputs minus the pulmonary blood flow. Its value in the model, scaled for the lamb, is 444 $ml \cdot min^{-1} \cdot kg^{-1}$. A 64% decrease in combined cardiac output to 283 $ml \cdot min^{-1} \cdot kg^{-1}$, with no change in blood pressure, flow distribution, or oxygen consumption, is the minimum value compatible with adequate oxygen delivery. However, under these circumstances, in some compartments venous blood oxyhemoglobin saturation is 0%. To obtain venous oxyhemoglobin saturations of 5% or above (corresponding to a venous Po_2 of 8 mm Hg or higher), the cardiac output has to be above 303 $ml \cdot min^{-1} \cdot kg^{-1}$. To obtain a oxyhemoglobin saturation of 10% (Po_2 of 10 mm Hg), a cardiac output of 324 $ml \cdot min^{-1} \cdot kg^{-1}$, 74% of the normal value, is required. The critical organs of observation are the brain and myocardium. It should be stressed here that the model does not include a possible change in myocardial oxygen consumption or redistribution of blood flow.

The computed boundary values for normal oxygen delivery depend on fetal hemoglobin concentrations. Fig. 2 shows the predicted boundaries for the combined cardiac output as a function of fetal hemoglobin concentrations. The fetal hemoglobin level in the sheep is reported to be between 9 and 12 gm/100 ml; a value of 11 gm/100 ml was used in the model. Fig. 2 shows that the normal human fetal hemoglobin level, that is, between 15 and 20 gm/100 ml, is compatible with lower cardiac outputs as far as critical limits for minimum oxygen consumption are concerned. Table II gives values of flows and oxygen tensions for the model in three cases: a fetal lamb, a fetal lamb with a cardiac output comparable to that of the human, and a fetal lamb with both cardiac output and hemoglobin concentration scaled to human values. This table illustrates the compensatory effect of the increase in hemoglobin concentration.

Cardiac output, hemoglobin, and viscosity. The change in cardiac output with maintenance of normal blood pressure, distribution of flow, and volumes examined in our model has been observed in experiments studying the effect of change in blood viscosity in adult[9] and fetal[11] animals. These experiments suggest that the cardiac output is inversely proportional to the blood

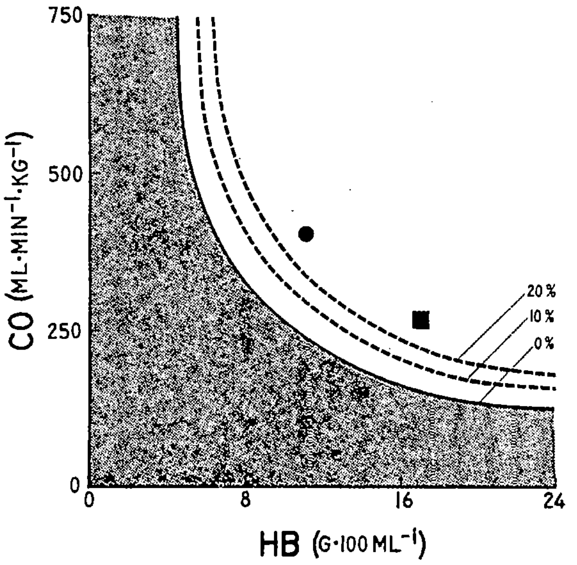

Fig. 2. Interrelations of combined cardiac output *(CO)* (sum of ventricular output minus pulmonary flow), in ml · min^{-1} · kg fetal $weight^{-1}$, and hemoglobin concentration *(HB)* and oxygen delivery. The *shaded area* shows where normal oxygen delivery is compromised. The *solid line* indicates the combinations of cardiac output and hemoglobin where in some organs all available oxygen is used in order to provide a normal oxygen consumption. *Dashed lines* indicate combinations where the venous blood of every organ contains at least 10% or 20% of its capacity. The normal values for the lamb (●) and human (■) are shown.

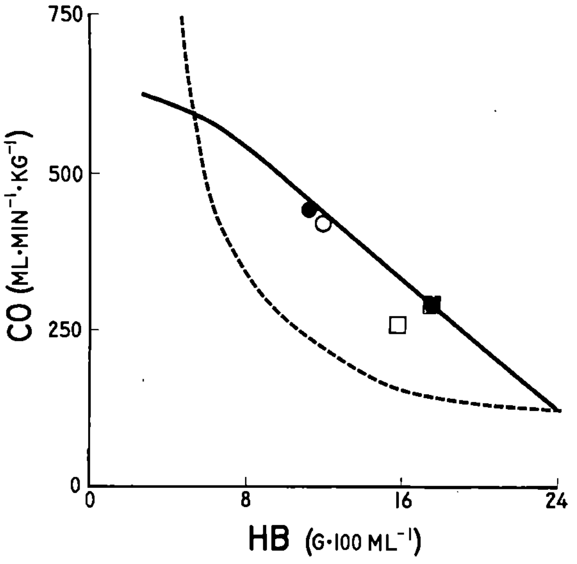

Fig. 3. Effect of viscosity on combined cardiac output *(CO)* (sum of ventricular outputs minus pulmonary flow) in ml · min^{-1} · kg fetal $weight^{-1}$. The *solid line* indicates changes in normal human value (■) due to changes in viscosity. The interrupted line indicates the boundary combinations of combined cardiac output and fetal hemoglobin *(HB)* for adequate oxygen delivery (see Fig. 2). Values for the baboon[15] (□), rhesus monkey[14] (○), and sheep[2] (●) are shown.

viscosity. In general, the viscosity effect on cardiac output is explained only by its effect on peripheral vascular resistance.[12] However a study using our model indicates that an increase in the peripheral resistance alone cannot explain the experimentally observed fall in cardiac output concomitant with no change in the arterial blood pressure.[9, 11] A direct effect on the performance of the heart itself in addition to the increase in peripheral resistance has to be assumed. The similarity between the observed experimental effects of viscosity changes and the changes made in our model to study differences in cardiac output suggests that differences in fetal cardiac output may result directly from changes in viscosity.

Blood viscosity is mainly determined by the hematocrit and to a lesser extent by the plasma proteins. Several studies indicate only minor differences in the viscosity of fetal and maternal blood of species with the same hematocrit.[10, 13] We computed the influence of hemoglobin concentration on cardiac output by assuming that the latter is inversely related to the viscosity, mediated by an increase in peripheral resistance together with a decrease in cardiac performance as discussed before. Fig. 3 presents the results of these computations together with reported values of fetal hemoglobin concentrations and combined cardiac output for different species. The data for rhesus monkeys[14] and baboons[15] are from acute experiments in which the fetus was in utero, comparable with early sheep studies.[16] Fig. 3 shows that the computed relationship between fetal cardiac output and hemoglobin concentrations depicted in Fig. 2 and the predicted changes due to viscosity are in agreement for hemoglobin values between 8 and 20 gm/100 ml. Thus the fetal cardiac output differences between sheep and humans not only are efficient as far as oxygen delivery is concerned but might be expected from the difference in viscosity. The observed decrease in human umbilical flow[5] and cardiac output[4] per kilogram of fetal weight, coupled with the fact that the human fetal hemoglobin concentration rises at the end of pregnancy, agrees well with this concept.

One may question what happens when a human fetus becomes anemic, as may occur in conditions such as Rh disease, α-thalassemia, and twin transfusion syndrome. The model predicts that the maintenance of adequate oxygen delivery requires an increase in fetal cardiac output; this increase can be effected by a decrease in fetal blood viscosity. The increase in fetal cardiac output due to a decreased hemoglobin concentration is substantial and should be readily detectable by ultrasound techniques. We therefore predict that the relationship between hemoglobin concentration and cardiac output may provide a clinically relevant diagnostic tool in the detection of fetal anemia. It should be noted, however,

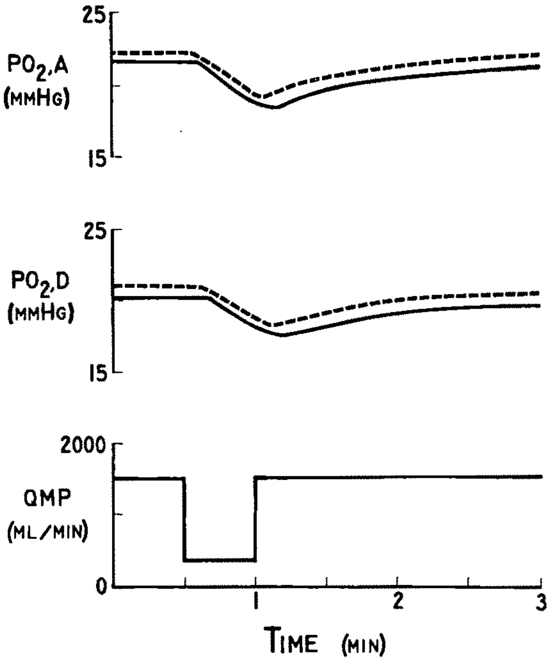

Fig. 4. Predicted responses of oxygen tension in the ascending ($\mathrm{Po_2}$,A) and descending ($\mathrm{Po_2}$,D) aorta to a decrease in maternal placental blood flow *(QMP)* in the human *(solid lines)* and the sheep *(dashed lines)* fetus.

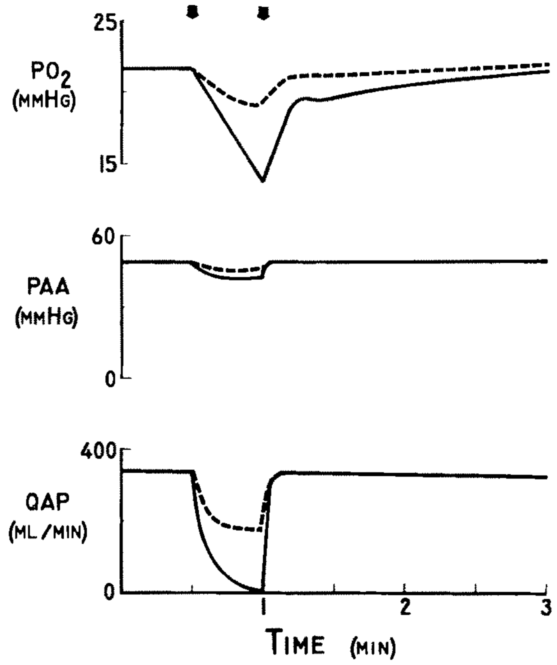

Fig. 5. Predicted responses of the fetal ascending aortic oxygen tension ($\mathrm{Po_2}$,) and blood pressure *(PAA)* and umbilical flow to the placenta *(QAP)* to a 30-second partial *(dashed lines)* and total *(solid lines)* obstruction in the umbilical vein. The beginning and end of the obstruction is indicated by *arrows*.

that in severe prolonged fetal anemia the increased cardiac output may be lessened by an increase in hepatic resistance due to extreme extramedullary hematopoiesis.[17]

Oxyhemoglobin dissociation curves. To study the effects of other parameter differences, we adjusted the model to the human by increasing the fetal hemoglobin level to 17 gm/100 ml and lowering the combined cardiac output to 279 ml · $\mathrm{min^{-1}}$ · $\mathrm{kg^{-1}}$.[4]

Differences in blood oxygen affinity can also affect fetal oxygen delivery. The human fetal and maternal oxyhemoglobin dissociation curves are different from those in the sheep. The curves used in our model have the form:

$$\log P = k_1 + k_2 \times \log (S/100 - S)$$

where P is the oxygen tension in millimeters of mercury, S is the percent of oxyhemoglobin saturation, and k_1 and k_2 are constants, $k_1 = 1.44$ and $k_2 = 0.39$ for human maternal blood and $k_1 = 1.32$ and $k_2 = 0.39$ for fetal blood.[18, 19] These curves are closely approximated in humans in vivo, which produces a limit for adequate placental oxygen transfer. The model predicts that a change from the sheep to human dissociation curves is incompatible with normal oxygen delivery to the fetal organs, even after the maternal hemoglobin level is adjusted from 10 to 12 gm/100 ml and the maternal oxygen saturation from 85% to 90% (human values). To obtain normal oxygen delivery to the fetus with arterial oxygen tensions comparable to fetal lamb values, a doubling of overall placental oxygen diffusing capacity (from 0.83 to 1.66 ml · $\mathrm{min^{-1}}$ · mm $\mathrm{Hg^{-1}}$ · kg fetal $\mathrm{weight^{-1}}$) is necessary. The overall placental diffusing capacity incorporates factors for uneven distribution, shunting, and other influences of the spatial arrangement of the exchange vessels. A more detailed model is needed to study placental differences between humans and sheep. The results of steady-state values for compartmental blood pressure and oxygen tension and central blood flows incorporating all these changes are given in Fig. 1.

Dynamic responses. Under these conditions we simulated a 75% decrease in maternal placental blood flow for 30 seconds. Fig. 4 shows the predicted changes in the oxygen tension in the fetal ascending and descending aorta. Because of differences in circulation time the predicted delay between the onset of the disturbance and the fall in $\mathrm{Po_2}$ is slightly longer in humans. In sheep experiments a 20-second decrease in uteroplacental blood flow is followed by a bradycardia with a time delay of 10 to 20 seconds, depending on the level of fetal oxygenation prior to the disturbance.[20] In the model's results, the time delay between the decrease in maternal blood flow and the observed fall in fetal arterial oxygen tension is comparable.

Fig. 5 shows the model's response of fetal arterial oxygen tension to a 30-second total, and partial, occlusion of the umbilical vein. These predicted responses are comparable with reported sheep experimental data[21]: The reported decrease in ascending aortic P_{O_2} from 22 to 18 mm Hg after 40 seconds of a 50% decrease in umbilical flow agrees with the model's prediction. The reported ascending aortic P_{O_2} after 40 seconds' total obstruction is 11 mm Hg, also compatible with the model's predicted decrease to 14 mm Hg in 30 seconds. This uncontrolled model with no feedback mechanisms predicts a slight fall in fetal arterial blood pressure. This response may differ when reflexes are taken into account.

The response of fetal arterial oxygen tension to a temporary decrease in maternal placental blood flow is comparable with the decrease in fetal heart rate seen in late decelerations. Likewise, the response to a temporary decrease in umbilical venous flow corresponds with the observed fall in fetal heart rate in variable decelerations. This suggests that both late and variable responses in fetal heart rate are mediated through a fall in oxygen tension.

Comment

Many results from experiments in laboratory animals are not directly transferrable to human physiology. The apparently low fetal cardiac output measured with ultrasound in humans has raised questions concerning the reliability of the technique. Because of this, simultaneous measurements in animals using ultrasound and direct methods need to be conducted. Nonetheless, our theoretical study predicts that the difference in fetal cardiac output between humans and sheep may be real and related to the difference in fetal hemoglobin concentrations. It must be stressed, however, that many parameter values in the model, of necessity, are based on laboratory experiments with sheep. The model embodies the blood distribution found in fetal sheep and the distribution in humans may be different. In particular, flow to the much larger human brain should be greater, although studies in sheep and primates indicate that blood flow per unit of brain mass is fairly constant in different species.[14, 15]

We conclude that the mathematical model is useful in providing a theoretical understanding of the differences between the sheep and the human fetus. This explanation includes a basis for the agreement between sheep and human oxygen delivery and responses to disturbances of it. However, it must be stressed that the higher human hemoglobin concentration only compensates for the lower cardiac output for substrates transported by red blood cells. This implies that several ratios of substrates would have substantial interspecies differences. We also speculate that fetal anemia should be detectable and measurable by the ultrasound technique. Finally, we believe that an extension of the model to include control of heart rate through chemoreceptor and baroreceptor reflexes may provide a tool to explain further the behavior of the fetal heart rate in humans.

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Intrauterine growth retardation induced by thiamine deficiency and pyrithiamine during pregnancy in the rat

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The role of nutritional factors in the development of prenatal and postnatal growth retardation is not well understood. We tested if thiamine deficiency may cause intrauterine growth retardation in rats. From the second day of gestation Sprague-Dawley rats were freely fed either a nutritionally complete or a thiamine-deficient diet. A similar group of rats was pair-fed with a complete or a thiamine-deficient diet and daily pyrithiamine injections (50 μg/100 gm of body weight) were given to precipitate thiamine deficiency during the short gestation of the rat. Maternal thiamine levels in blood and brain tissues, maternal erythrocyte transketolase activity with thiamine pyrophosphate effects, and fetal tissue thiamine levels were measured. The results indicate that feeding a thiamine-deficient diet in conjunction with pyrithiamine injections caused sufficient thiamine deficiency to induce intrauterine growth retardation in the progeny. We conclude that thiamine deficiency alone during in utero development in the rat may contribute to intrauterine growth retardation. (AM J OBSTET GYNECOL 1985;151:455-60.)

Key words: Intrauterine growth retardation, thiamine deficiency, fetal alcohol syndrome

Intrauterine growth retardation (IUGR), a characteristic feature of fetal alcohol syndrome,[1,2] is one of the major causes of prenatal mortality. However, the precise mechanism by which alcohol causes IUGR remains obscure. Alcohol itself may be harmful to the developing fetus by transient impairment of umbilical circulation,[3] but it is also conceivable that various side effects of ethanol metabolism, such as the production of acetaldehyde, ketones, and hypoglycemia, contribute to fetal alcohol syndrome.[4-6] Furthermore, chronic alcohol abuse is frequently associated with nutritional deficiencies. For example, alcohol directly interferes with the active transport of thiamine from the gut.[7] Ideally, one would like to determine the contribution of the direct and indirect toxic effects of ethanol in causing IUGR and fetal alcohol syndrome. However, such a model system is unavailable at present. It is entirely possible that the toxic effects of ethanol are much more pronounced in the fetus when a nutritional deficiency state is reached in the mother.

The present investigation was, therefore, undertaken to determine the contribution of nutritional factors, such as thiamine deficiency alone, as a cause of IUGR. Thus in this model one would be able to study an isolated nutritional component (for instance, thiamine deficiency) separately from the direct toxic effects of ethanol.

Here, we report that thiamine deficiency alone during pregnancy in the rat causes fetal growth retardation and may be a major contributing factor in the production of IUGR in fetal alcohol syndrome. The parameters indicative of IUGR included decreased fetal body weight and disproportionate decreases in fetal brain and liver weights which resulted in a higher brain/liver ratio.[8]

Material and methods

Sprague-Dawley rats (9 weeks old, from the second postcoital day) were used for all experiments (Taconic Farms, New York). Diets were obtained from Bio-Serv Inc., Frenchtown, New Jersey. Control and thiamine-deficient diets contained 4.4 and 0.4 mg of thiamine per kilogram of diet, respectively. Pyrithiamine hy-

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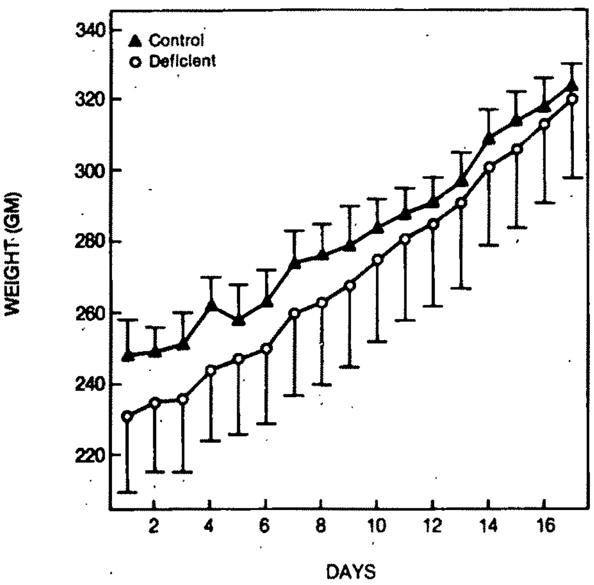

Fig. 1. Maternal weight changes during gestation in group 1 dams. Note similar rate of weight gain. Values represent means ± SEM.

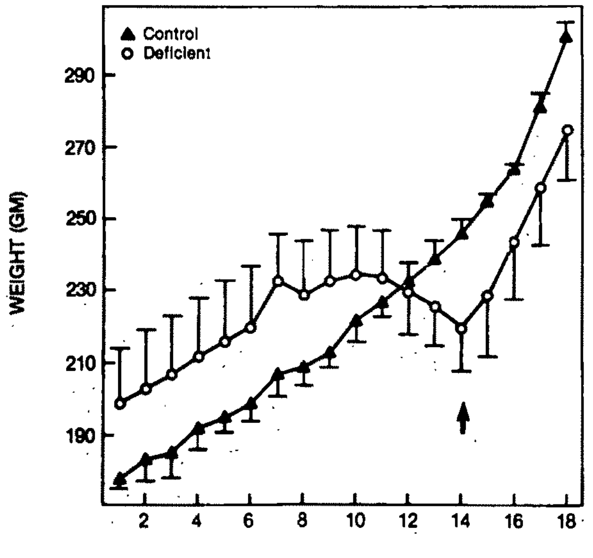

Fig. 2. Maternal weight changes during gestation in group 2 dams. Note that dams given thiamine-deficient diets and daily pyrithiamine injections lost weight within 8 to 10 days compared to control animals that continued to gain weight throughout gestation. Values represent means ± SEM. *Arrow* represents day of injection of subcutaneous thiamine and return of experimental animals to control diet with an ensuing weight gain.

drobromide and thiamine hydrochloride were supplied by Sigma Chemical Co., St. Louis, Missouri.

The rats were housed under standard laboratory conditions with periods of 12 hours light/12 hours dark and weighed daily. Wire-bottom cages were used to prevent coprophagia and water was given as desired. Dams were divided into four groups and put on regimens as follows: group 1, thiamine-deficient versus control diet as desired; group 2, same as group 1 but dams also received daily subcutaneous injections of a thiamine antagonist, pyrithiamine; group 3, same as group 2 except that dams were pair-fed rather than provided with diet as desired; group 4, control diet with daily pyrithiamine injections versus pair-fed controls receiving saline solution injections. Pyrithiamine was given in a dose of 50 μg/100 gm of body weight to precipitate thiamine deficiency.[9] Rats were considered to be thiamine deficient when overt signs of encephalopathy, for instance, ataxia, loss of righting reflexes, opisthotonus, and seizures, were observed. At this point, pyrithiamine injections were stopped, and the animal was injected with subcutaneous thiamine at a dose of 100 mg per kilogram of body weight[9] and returned to a control diet for the remainder of the experimental period.

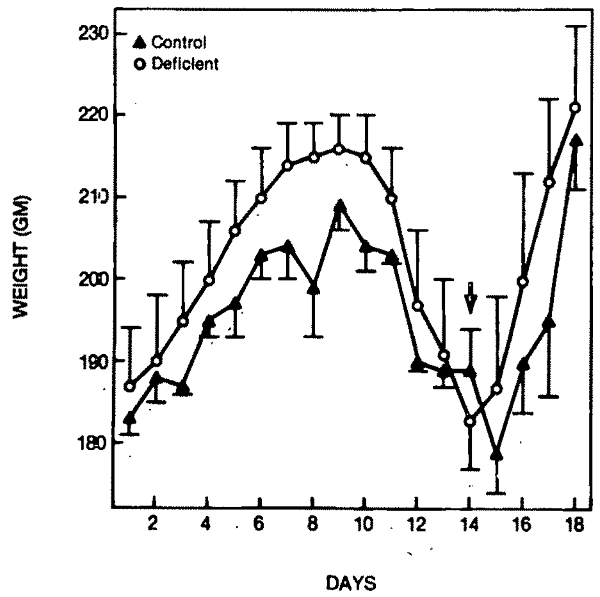

Fig. 3. Maternal weight changes during gestation in group 3 dams. Note that these dams showed similar patterns of weight change because of pair-feeding. Values represent means ± SEM. *Arrow* represents day of reconstitution with subcutaneous thiamine and return of experimental animals to control diet with an ensuing weight gain.

Rats were put to death by decapitation on the nineteenth day of gestation (term, 22 days) and maternal serum was collected and kept on ice for glucose analysis by a Beckman glucose analyzer. Fetuses were randomly removed by cesarean section from both uterine horns. Fetal body weight, placental weight, brain weight, and liver weight were recorded, and fetuses examined for any gross malformation.

To determine maternal and fetal thiamine levels, dams receiving a thiamine-deficient diet and daily pyrithiamine injections were put to death at day 13 to 15 of gestation. During this time period the rats became

Table I. Maternal and fetal thiamine levels and transketolase activity with thiamine pyrophosphate effect

	Thiamine levels			*Transketolase activity (nmol/min/mg protein)*		
Diet	*Maternal blood (μg/100 ml)*	*Maternal brain (μg/gm protein)*	*Fetal homogenate (μg/gm protein)*	*Without thiamine pyrophosphate*	*With thiamine pyrophosphate*	*Effect of thiamine pyrophosphate (%)*
Control, at will	12.7 ± 1.2	29.4 ± 2.4	38.5 ± 11.2	12.8 ± 0.1	12.0 ± 0.7	0
Thiamine deficient and pyrithiamine	3.0 ± 0.6	4.3 ± 1.1	3.48*	5.09†	8.57†	68
Pair-fed control, thiamine deficient plus pyrithiamine	4.5 ± 1.15	8.64 ± 0.56	6.14*	8.44†	8.19†	0

Values represent the mean ± SD.
*Sole value obtained in this category.
†Average of two determinations.

Table II. Weight comparisons—fetal parameters

	Body weight (gm)		*Placental weight (mg)*		*Brain weight (mg)*		*Liver weight (mg)*		*Brain/liver ratio*	
Group	*Control*	*Experimental*	*Control*	*Experimental*	*Control*	*Experimental*	*Control*	*Experimental*	*Control*	*Experimental*
1	2.52 ± 0.21	2.36 ± 0.30	475 ± 60	470 ± 95	110 ± 15	115 ± 13	148 ± 31	131 ± 25	0.79 ± 0.25	0.90 ± 0.17
	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
2	1.61 ± 0.39	1.30 ± 0.25	466 ± 90	381 ± 56	99 ± 14	75 ± 15	115 ± 30	81 ± 26	0.92 ± 0.31	1.0 ± 0.37
	$p < 0.05$		$p < 0.01$		$p < 0.01$		$p < 0.01$		$p > 0.05$	
3	1.29 ± 0.17	1.13 ± 0.25	387 ± 70	316 ± 71	73 ± 13	63 ± 21	78 ± 22	50 ± 18	1.0 ± 0.30	1.38 ± 0.67
	$p < 0.01$		$p < 0.01$		$p > 0.05$		$p < 0.01$		$p < 0.05$	
4	1.42 ± 0.15	1.47 ± 0.11	436 ± 82	418 ± 69	92 ± 16	95 ± 6	103 ± 19	100 ± 13	0.90 ± 0.17	0.96 ± 0.13
	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	

Values represent mean ± SD.
*Significance of data was tested by two-tailed *t* test.

ill enough to require subcutaneous thiamine reconstitution. The appropriate pair-fed dams receiving a control diet and daily pyrithiamine were also put to death simultaneously. Samples of maternal whole blood and maternal brain homogenates were analyzed for thiamine levels (Bio-Science Laboratories, Columbia, Maryland). Aliquots of maternal blood were also processed for assay of transketolase activity and related thiamine pyrophosphate effect according to established procedure.[10] Moreover, fetuses from thiamine-deficient and from control mothers were homogenized to determine thiamine levels in fetal tissues. Total protein in maternal and fetal tissue homogenates was determined by the method of Lowry et al.[11] and the thiamine levels were normalized for all tissues.

Data (see Table II) were analyzed by two-tailed Student's *t* test[12] between control and treated groups at different fetal parameters for each treatment group.

Results

Maternal parameters. Animals in group 1 exhibited no difference in weight gain throughout the gestational period (Fig. 1). In group 2 the animals on a thiamine-deficient diet and receiving pyrithiamine lost weight within 8 to 10 days whereas control animals continued to gain weight throughout gestation (Fig. 2). Experimental animals in group 3 demonstrated a similar decrease in weight, but because of pair-feeding the weights of control animals also decreased (Fig. 3). When dams on a thiamine-deficient diet in groups 2 and 3 showed signs of encephalopathy, they received subcutaneous thiamine injections and returned to a nutritionally complete diet. A subsequent weight gain was observed within 48 hours of reconstitution.

Representative thiamine levels obtained at the peak of illness secondary to thiamine deficiency are shown in Table I. As indicated, maternal blood and brain thiamine levels from animals receiving a thiamine-deficient diet plus pyrithiamine are approximately 25% and 15%, respectively, of those found in a dam receiving a control diet at will. Maternal erythrocyte transketolase activity was also measured. As demonstrated in Table I, maternal blood from thiamine-deficient dams showed low activity with a marked thiamine pyrophosphate effect that averaged 68%. A thiamine pyrophosphate effect is generally regarded as an index of thiamine deficiency.[13] No such thiamine pyrophosphate effect was demonstrated for dams on a control diet.

Maternal glucose levels determined at the time of sacrifice were not significantly different between thiamine-deficient and control dams (p = NS) for all groups investigated.

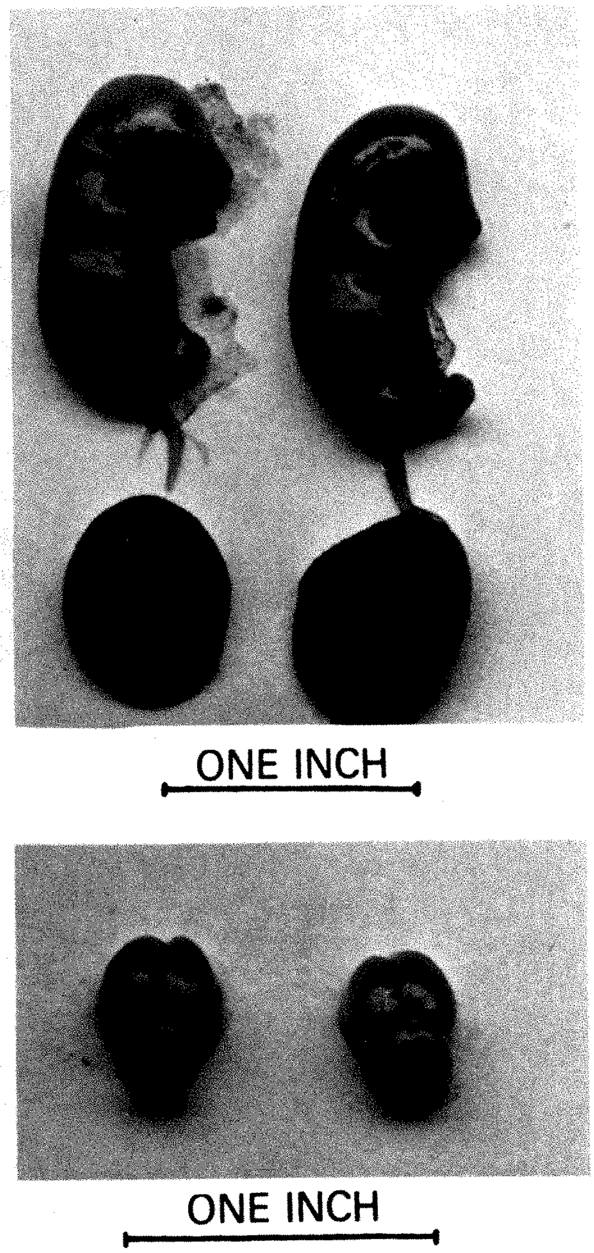

Fig. 4. Comparison of fetuses, placentas, and brains. Composite pictures show similarity of experimental and control fetuses in group 4. *Left:* Experimental; *Right:* control.

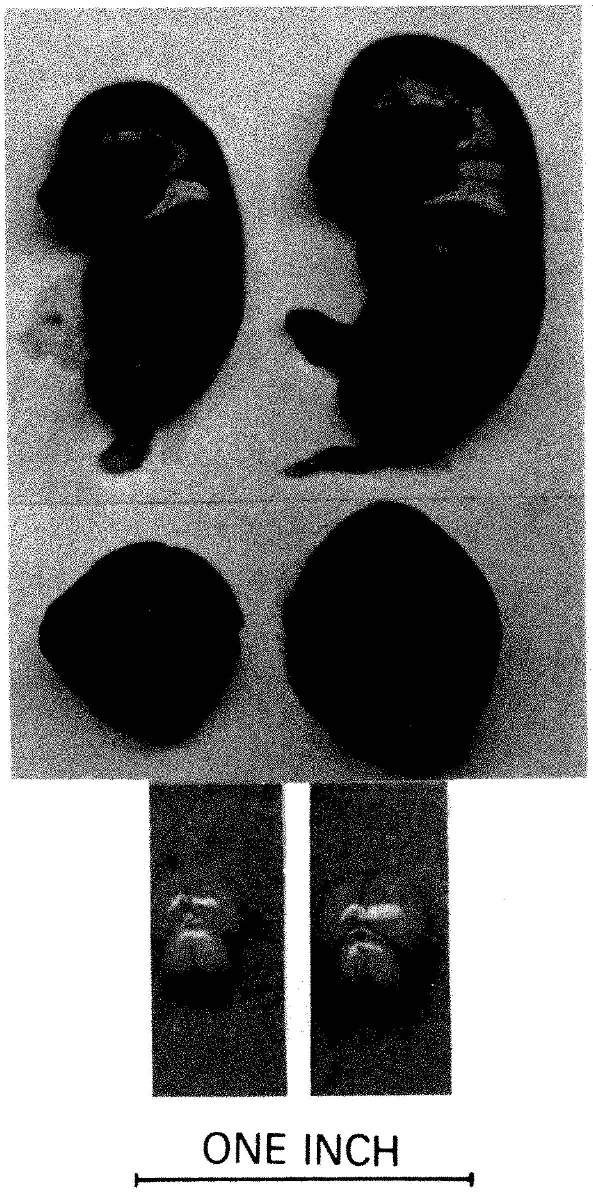

Fig. 5. Comparison of fetuses, placentas, and brains. Composite picture on the left represents fetus born of a mother given a thiamine-deficient diet and pyrithiamine during gestation. Composite picture on the right represents fetus born to a control mother.

Fetal parameters. The effect of thiamine deficiency on fetal body weight, placental weight, brain weight, liver weight, and brain/liver ratio is presented in Table II.

The fetal parameters recorded for groups 1 and 4 demonstrated no significant differences ($p > 0.05$). Fig. 4 is a composite picture showing the similarity of experimental and control fetuses from group 4 in terms of body size, placental size, and brain size.

Experimental fetuses from group 2 did show significant differences when compared to control fetuses. The mean difference in body weight was significant at the $p < 0.05$ level, whereas mean differences in placental, brain, and liver weights were significant at $p < 0.01$. The mean difference in brain/liver ratio was not found to be significant ($p > 0.05$).

For group 3 the mean differences in weight of fetal body, placenta, and liver as well as brain/liver ratios were significant. Fig. 5 illustrates the differences between a fetus from an experimental mother and one from a pair-fed control mother.

Further information regarding group 3 fetuses is provided in Table I. As shown, the thiamine level of fetal homogenate derived from a dam on a thiamine-deficient diet and pyrithiamine was approximately 10% of the value obtained from control fetuses and one half the level found in pair-fed control fetuses.

No fetuses of any of the groups investigated displayed obvious dysmorphic features.

Comment

Thiamine deficiency induced by a thiamine-deficient diet plus pyrithiamine causes IUGR in the rat. However, a thiamine-deficient diet alone is not enough to precipitate significant deficiency of this vitamin in the rat within its short gestational period. This was demonstrated by the fact that rats fed a thiamine-deficient diet in group 1 displayed no maternal weight loss and showed no signs of encephalopathy. Additionally, the fetuses of these animals showed no evidence of IUGR. It has recently been reported that thiamine-deficient female rats do not reproduce.[14] This excluded the use of animals in which thiamine deficiency was induced prior to pregnancy. Therefore, for our study we induced thiamine deficiency in the dams after successful mating by using a thiamine antagonist, pyrithiamine. This structural analogue of thiamine produces classical symptoms of thiamine deficiency during a relatively short period of time by reducing thiamine pyrophosphate availability in tissues. Furthermore, pyrithiamine enhances excretion of thiamine in urine and prevents thiamine pyrophosphate from combining with enzyme proteins[15] where it serves as a coenzyme. As a result of pyrithiamine's antagonism of thiamine, combined with a deficient diet, rats in groups 2 and 3 experienced weight loss after 8 to 10 days and showed signs of encephalopathy after 12 to 14 days. Without pyrithiamine it generally takes 4 to 5 weeks for rats to become ill on a thiamine-deficient diet alone,[16] which is much longer than a rat's gestational period.

The fetuses of the rats receiving a thiamine-deficient diet at will and daily pyrithiamine injections (group 2) demonstrated characteristics of IUGR as previously described.[8] However, these findings did not take into account the severe maternal weight loss of the experimental rats compared to the control rats. Therefore, to eliminate malnutrition as a variable, pair-feeding was used to normalize the weight differences as observed in group 2.

The fetuses of dams in group 3 demonstrated characteristics of IUGR. Evidence that these experimental dams became thiamine deficient has been provided (Table I). Thiamine-deficient status was, therefore, established on both clinical and biochemical grounds. An absolute value for fetal tissue thiamine was also obtained, indicating that maternal thiamine deficiency is indeed transmitted to the developing fetuses. Finally, to ensure that the data obtained were due only to the relative thiamine deficiency induced by pyrithiamine and not to any toxic effects of pyrithiamine, we studied group 4. This group demonstrated no significant differences between the means of the parameters investigated. The results of the experiment suggest that pyrithiamine alone does not cause IUGR.

We therefore feel that a lack of thiamine during early development induces IUGR in rats. The reduced body weight, placental weight, and liver weight are indicative of IUGR and the higher brain/liver ratio (that is, "brain sparing") is consistent with IUGR seen in man.[17]

As in all experimental situations, extrapolation of data from animal models to humans should be done with caution. Nonetheless, our rat model may enable one to gain insight into the mechanisms by which nutritional factors may induce IUGR in fetal alcohol syndrome.

We thank Mr. Richard Staton for his assistance in statistical analysis of the data.

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Ovine placental vascular response to the local application of prostacyclin

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Rankin et al. have shown that prostacyclin does not dilate the ovine placenta, yet Clark et al. have shown that retrograde infusion of prostacyclin dilates the uterine vasculature. To determine the effects of prostacyclin on the sheep placenta, the two groups have collaborated. In five chronically catheterized near-term sheep, blood flows were measured by the radioactive microsphere technique. Control blood flows were measured and prostacyclin infused into the retrograde uterine artery catheter at 10 μg/min. The blood flows were again measured, and the area served by the catheter was then delineated by the injection of a green dye at the time of maternal euthanasia. The placenta and cotyledons were separated and the kidneys were obtained for assay. The systemic effects of prostacyclin were minimal and have been observed previously. The resistance of the myometrium fell from 566 ± 99.9 to 322 ± 63.9 mm Hg · ml^{-1} · min · gm ($p < 0.002$). The placental tissue showed no change in resistance. When the tissues were combined, we observed a decrease in resistance from 0.38 ± 0.18 to 0.32 ± 0.14 mm Hg · ml^{-1} · min ($p < 0.004$), thereby confirming the observation that there was indeed vasodilation downstream. However, the vasodilation was not in the placenta but in the myometrium, and we reconfirm our previous conclusion that prostacyclin does not dilate the sheep placenta. (AM J OBSTET GYNECOL 1985;151:460-4.)

Key words: Prostacyclin, placental vasculature, microsphere technique, retrograde infusion

The effect of prostacyclin on uterine and placental blood flow has been investigated in many species including the dog, sheep, rat, and human. Prior work by Clark et al.[1, 2] has shown prostacyclin to be a potent uterine vasodilator in the nonpregnant sheep and to produce a 28% increase in blood flow to the pregnant sheep uterus when measured with an electromagnetic flowprobe on the uterine artery. This is not in agreement with prior work by Rankin et al.[3] who have shown that prostacyclin increases placental vascular resistance when infused systemically to the pregnant ewe.

In order to resolve these apparent differences, we have collaborated in a joint study to examine the effects of maternal prostacyclin infusion on the maternal uterine placental and nonplacental vascular beds in the pregnant ewe.

Material and methods

The protocol and preparation of Clark et al.[1, 2] were reproduced, in that maternal drug administration was via retrograde uterine catheter at a dose of 10 μg/min of prostacyclin. Measurements of blood flow were made by analysis of microsphere distribution in order to differentiate uterine placental and nonplacental changes in blood flow. Five sheep of between 120 and 130 days of gestation were used.

Surgical procedures. Surgery was performed under ketamine anesthesia, 8 mg intramuscularly and 0.1 mg/ml intravenous drip, with supplemental Nembutal as needed. The animals were not given food for 48 hours, and water was withheld for the day preceding surgery. Both maternal femoral arteries were catheterized, as

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was the left jugular vein and left ventricle. Midline abdominal incisions were made to expose the uterine vessels, and a retrograde catheter was placed in a lateral branch of the maternal middle uterine artery and threaded back to the point of branching of the artery. A small uterine incision was made, and fetal hindlimb vein and arterial catheters were placed. The uterus was oversewn and all catheters brought out through the flank to a small cloth pouch on the animal's left side. Sheep were allowed a postoperative recovery period of 3 to 4 days. These procedures have been previously described by Buss et al.[4] and Clark et al.[2]

Experimental procedures. All experiments were done on postoperative day 3 or 4. The ewe was allowed to acclimate to a stanchion while blood pressure and pulse were recorded via a pressure transducer placed at the level of the scapulohumeral joint.

Prostacyclin was prepared within 1 hour of the experiment from frozen stock solution in 0.05 mol/L Tris buffer, diluted in fresh Tris buffer. Clark et al.[2] used a solution made fresh from crystaline sodium prostacyclin and dissolved in 0.01 mol/L Tris base buffer with a pH of 9.4. The prostacyclin was supplied by the Upjohn Company. A 5-minute control infusion of buffer solution was given via the retrograde uterine catheter followed by a microsphere injection into the left ventricle. Blood withdrawal, at 4.12 ml/min via the femoral artery catheter, continued for 2 minutes after the microsphere injection. A 5-minute test infusion of 10 μg/min of prostacyclin was then given, and again microspheres were injected during the fifth minute. Fetal viability was established before and after each experiment by monitoring of fetal heart rate via the hindlimb catheter. The ewe was killed at the end of the experiment. Green dye was instilled via the retrograde uterine artery catheter to indicate which cotyledons and uterine tissue were exposed to the prostacyclin. Immediate laparotomy was performed, with removal of the uterus and contents and maternal kidneys.

The microspheres used (3M Co. and New England Nuclear) were 15 μm in diameter and labeled with either ^{141}Ce, ^{85}Sr, ^{125}I, or ^{46}Sc. Each injection consisted of 15 μCi of radioactivity in 0.40 ml of 10% dextran in normal saline solution and contained approximately 10^6 microspheres.

Measurements and tissue preparation. Measurements were made of maternal blood pressure and heart rate with a Statham P23Db transducer and cardiotachometer.

The tissues were processed following the experiment. Placental cotyledons were separated from fetal membranes and myometrium, weighed, and bottled. Green and nongreen myometrium were separated, weighed, and homogenized with measured amounts of water. Ten aliquots, approximately 3 ml in volume, were taken from each homogenate. The kidneys were also homogenized in similar fashion, and five aliquots were taken from each.

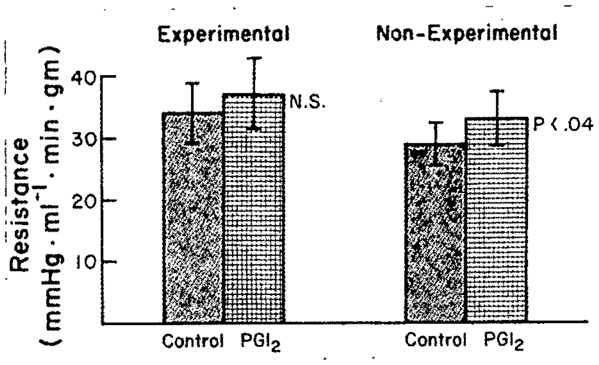

Fig. 1. The effects of prostacyclin *(PGI_2)* infusion on the resistance of placental tissues downstream from the retrograde catheter *(experimental)* and the tissue that was not downstream from the retrograde catheter *(nonexperimental)*.

Radioactivity was assayed by means of a Nuclear Chicago 1185 3-channel gamma counter. The number of spheres in each organ and the blood flow to that organ were determined using previously described methods.[4] Three isotopes were used, so the sample stream was preceded by three standards, each containing a known number of microspheres of each type used in the study. The spillover of each isotope in the other channels and the counts per minute per sphere were determined from the information obtained from the standard vials. Knowledge of the spillover of each isotope in the other channels and the counts per minute per sphere enabled us to calculate the number of spheres of each type in each vial. Blood flows were calculated by equations previously described.[4] The vascular resistance of an organ was defined as the maternal blood pressure divided by organ blood flow. Statistical analysis was by paired *t* test with logarithmic transformation of data where ratios were compared. We have previously tested the method of retrograde dye injection and microsphere localization in the placenta to ensure that there is good correlation between the area dyed and the presence of previously injected microspheres.

Results

Five sheep were used. All measurements are given as mean ± SEM. Tris (0.05 mol/L) buffer infusion was given during the control observations. The following results were obtained during the infusion of prostacyclin.

There was no significant change in blood pressure with control maternal arterial measurements of 78 ± 6 mm Hg and test measurements of 74 ± 4 mm Hg (not significant). The maternal heart rate did change significantly from a mean of 135 to 168 bpm with an

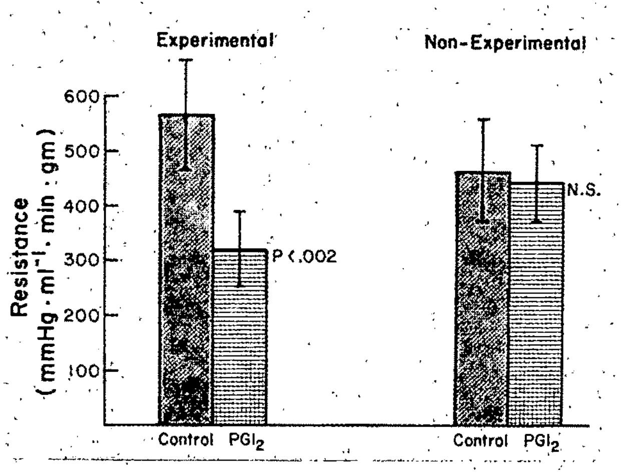

Fig. 2. The effect of prostacyclin infusion on the resistance of nonplacental (uterine) tissues downstream from the retrograde catheter *(experimental)* and the tissue that was not downstream from the retrograde catheter *(nonexperimental).*

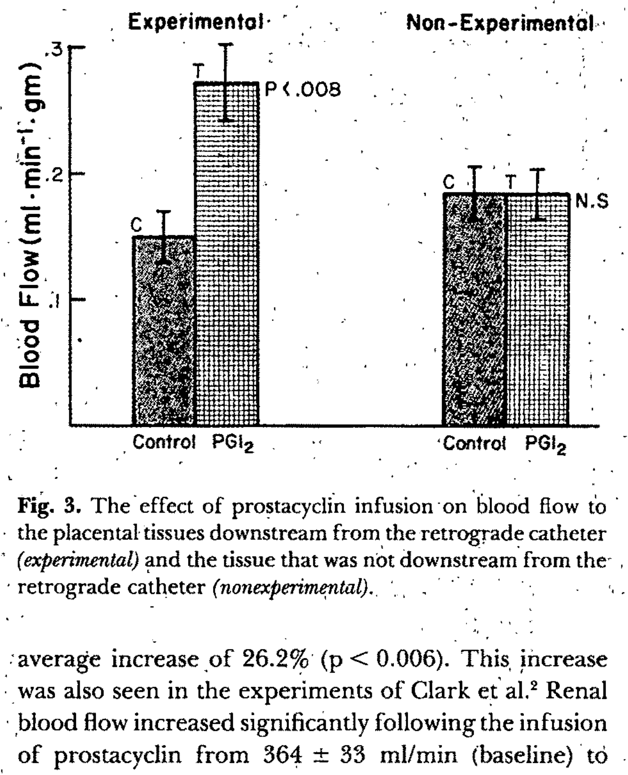

Fig. 3. The effect of prostacyclin infusion on blood flow to the placental tissues downstream from the retrograde catheter *(experimental)* and the tissue that was not downstream from the retrograde catheter *(nonexperimental).*

average increase of 26.2% ($p < 0.006$). This increase was also seen in the experiments of Clark et al.[2] Renal blood flow increased significantly following the infusion of prostacyclin from 364 ± 33 ml/min (baseline) to 431 ± 34 ml/min (test period), with $p < 0.003$. Renal vascular resistance fell from a mean of 0.24 ± 0.03 to 0.18 ± 0.02 mm Hg · min^{-1} · ml with $p < 0.002$.

The tissues that had been dyed green were used to calculate the experimental changes in blood flow and resistance in the placental and nonplacental uterine tissue. Nongreen tissues showed no changes in resistance, with an average experimental/control resistance ratio of 0.97 for the myometrium and 1.10 for the cotyledons. In Fig. 1 we compare the changes in resistance per gram of tissue in the experimental tissues to the results in the nonexperimental (contralateral side) tissues. In the nonplacental uterine tissue there was a drop in vascular resistance from 566 ± 99.9 mm Hg · ml^{-1} · min · gm to 322 ± 63.9 mm Hg · ml^{-1} · min · gm ($p < 0.002$), while the nonexperimental tissue showed no significant change in vascular resistance during the test situation with a control observation of 466 ± 93.5 mm Hg · ml^{-1} · min · gm and a test value of 447 ± 67.5 mm Hg · ml^{-1} · min · gm (not significant). The placental tissues showed no change in vascular resistance under test conditions in either the experimental or nonexperimental portions of the placenta, with calculated resistance of the vasculature within the distribution of the cannulated artery of 34 ± 5 and 37 ± 5 mm Hg · ml^{-1} · min · gm (not significant) and the nonexperimental tissue resistances of 29 ± 3.5 and 33 ± 3.9 mm Hg · ml^{-1} · min · gm in the control and test periods.

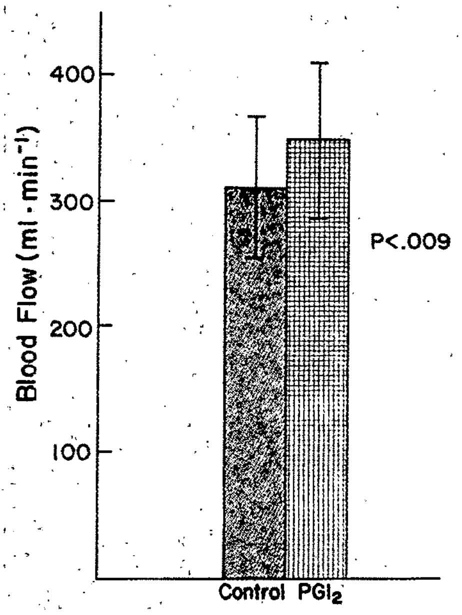

Fig. 4. The effect of administering prostacyclin on the blood flow in the vascular bed downstream from the retrograde catheter (experimental). This vascular bed consists of placental and nonplacental uterine tissue.

In the experimental tissues, nonplacental blood flow increased significantly in the test period, whereas placental blood flow showed no significant change. In Fig. 2 the changes in uterine nonplacental and placental blood flows are shown. The mean blood flow to nonplacental tissue during the prostacyclin infusion was 136 ± 25 ml/min (total flow) with a control value of 75 ± 12 ml/min ($p < 0.007$). The cotyledonary blood flow during the infusion remained constant at 236 ± 46 ml/min with a control value of 213 ± 45 ml/min (not significant).

If the total flow to both placental and nonplacental tissues is combined, however, a significant overall increase in blood flow is seen in this series and would have been detected by a flowprobe on the catheterized artery. The combined flow during infusion was 349 ± 64 ml/min compared to a control value of 311 ± 57 ml/min ($p < 0.009$), with a mean increase of 12%. The decrease in vascular resistance of the combined tissues in the experimental part of the uterus was also significant. The mean resistance in the test period is 0.32 ± 0.14 mm Hg · ml^{-1} · min, with control resistance of 0.38 ± .18 mm Hg · ml^{-1} · min ($p < 0.004$) (Figs. 3 and 4).

Comment

The experimental conditions and protocol described by Clark et al.[1,2] have been reproduced. We saw a similar change in heart rate with the prostacyclin infusion, with no significant change in blood pressure. In the present study prostacyclin was prepared in Tris buffer, frozen until used, and then further diluted in Tris on the day of the study. In the studies of Clark et al.[1,2] prostacyclin solutions were made from crystalline sodium prostacyclin in Tris (0.01 mol/L) on the day of infusion. Therefore there may have been some degradation in the present study to the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$. However, Clark et al.[2] have shown that this compound has minimal and variable activity on the ovine uterine vasculature, and it should not have had a significant effect on the results.

Our laboratory has extensive experience with the microsphere technique. The major source of error with this technique, too few microspheres in the integrated arterial or organ tissue samples, was avoided in that there were >400 microspheres in each sample.[5] All tissues were prepared carefully and counted in similar fashion, so that it would be unlikely that any counting or calculation error would have affected only the experimental tissues.

In our previously reported work,[6] catecholamines may have played a role in placental vascular responses because of hypotension resulting from the systemic administration of prostacyclin. In the series of experiments described here it would seem unlikely that this occurred because the drug was applied locally via the retrograde uterine catheter and blood pressure did not change.

The responses of the renal and uterine vasculature are consistent with published data in the sheep.[2,3,6,7] If we had looked at the myometrial and placental tissues as a whole, we would have concluded that vasodilation had occurred in the uterine vascular bed. With the microsphere technique we were able to separate placental from nonplacental tissue. It is clear that while there is decreased resistance in the nonplacental vascular bed, the placenta is unaffected, with no significant change in resistance or blood flow.

Uterine contractile activity is associated with decreased placental blood flow, but prostacyclin and Tris buffer (as vehicle) have been shown not to cause increases in either contractile activity or uterine tone.[2]

Clark et al.[2] have previously shown that a 28% increase in uterine artery blood flow occurred at a prostacyclin infusion rate of 10 μg/min. We show a mean change of 11.6%. There are several possible reasons for this discrepancy in the absolute value of the change. The amount of prostacyclin versus the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ may have been different, different breeds of sheep may have been used, and the animals may have been sampled at different times during the infusion.

Though it is difficult to apply results obtained with animal models to humans, there are case reports of prostacyclin administration to preeclamptic patients which are explicable with use of our model.[8,9] In both cases, prostacyclin infusions were begun in women with established preeclampsia. In the first case, severe fetal bradycardia developed despite improved maternal vital signs; in the second, fetal death occurred despite stable maternal parameters. There are many possible mechanisms by which the prostacyclin may have affected the fetoplacental unit in these instances, one of which being decreased placental perfusion.

It now seems well established that prostacyclin is decreased in maternal and fetal serum in states of chronic placental insufficiency.[10-18] The decreased levels of prostacyclin may be an effect rather than a cause of the vascular deficiencies seen in these states. It seems wise to follow established guidelines for the treatment of pregnancy-induced hypertensive disorders until the mechanisms of the decrease have been more clearly elucidated.

We wish to thank Dr. John Pike of the Upjohn Company for his help in providing the prostaglandins used in this work.

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Effect of dexamethasone treatment on sex steroid–binding protein, corticosteroid-binding globulin, and steroid hormones in cycling rhesus macaques

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We tested the hypothesis that dexamethasone lowers sex steroid–binding protein levels and observed the effect of dexamethasone on corticosteroid-binding globulin and specific steroid hormones in plasma. Four cycling rhesus macaques were studied during three consecutive menstrual cycles (first and third cycles served as controls). In the second cycle, each animal received 0.5 mg of dexamethasone intramuscularly at 8:00 AM and 8:00 PM daily for 21 consecutive days. Blood samples were taken at 1- to 3-day intervals during each cycle. Concentrations of sex steroid–binding protein, corticosteroid-binding globulin, cortisol, testosterone, progesterone, and estradiol were measured in plasma. The percentage and plasma concentration of free testosterone were also determined. Within 2 days of treatment, dexamethasone suppressed cortisol to 5% of baseline values, which returned in the third cycle. All cycles were ovulatory. Dexamethasone significantly lowered plasma levels of all the compounds except progesterone: sex steroid–binding protein, −30%; corticosteroid-binding globulin, −14%; testosterone, −36%; and estradiol, −45%. The percentage of free testosterone was significantly elevated, but free testosterone concentrations were unchanged. Although our data conclusively show that dexamethasone suppresses plasma sex steroid–binding protein levels in the rhesus macaque, it remains to be established whether this suppressive effect leads to an increase in the metabolic clearance rate of testosterone. (AM J OBSTET GYNECOL 1985;151:464-70.)

Key words: Sex steroid–binding protein, corticosteroid-binding globulin, dexamethasone, rhesus macaque

Administration of pharmacologic amounts of dexamethasone to female rhesus macaques results in a marked increase in the metabolic clearance rate of estradiol.[1] Studies also show that there is a rise in the metabolic clearance rate of estradiol and testosterone following administration of adrenocorticotropic hormone to women.[2] We were intrigued by the possibility that these findings may be explained by a mechanism of altered sex steroid–binding protein levels in plasma. Lowered concentrations of sex steroid–binding protein would result in an increase in the percentage of free estradiol and testosterone. Depending on the produc-

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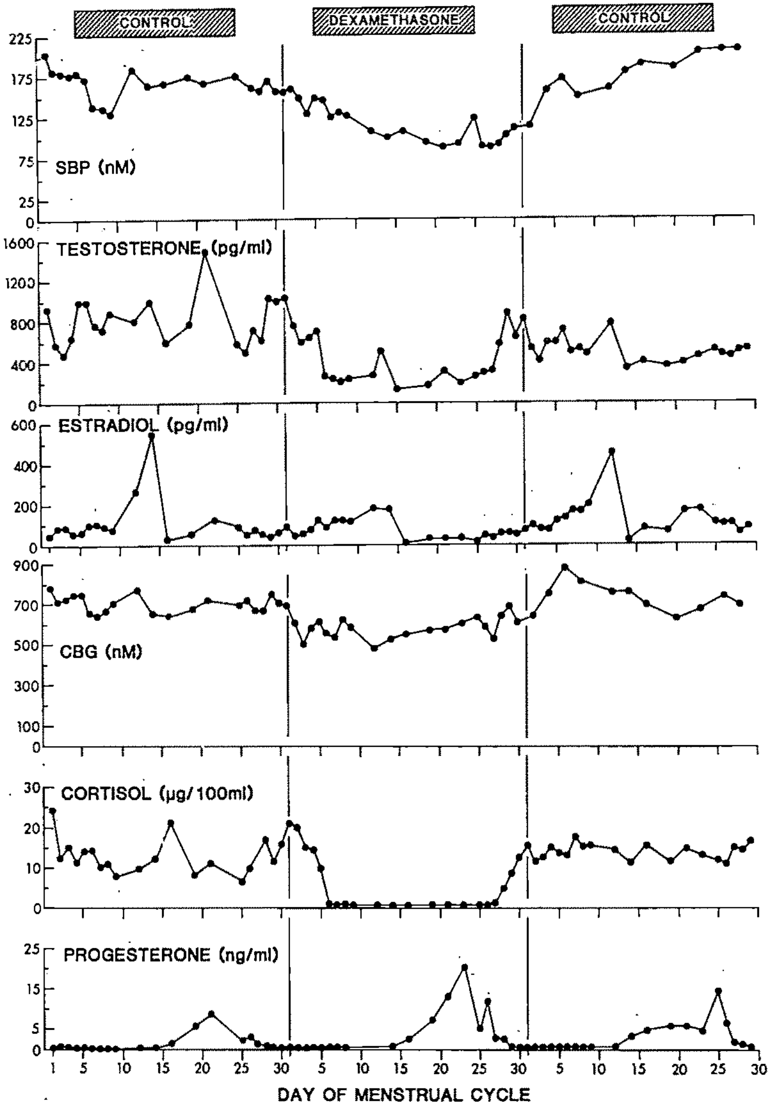

Fig. 1. Plasma levels of sex steroid–binding protein *(SBP)*, testosterone, estradiol, corticosteroid-binding globulin *(CBG)*, cortisol, and progesterone throughout three consecutive menstrual cycles of a representative study animal.

tion rate of these hormones, free estradiol and testosterone levels may also increase, resulting in an elevated metabolic clearance rate of the hormones.

The effect of dexamethasone on levels of circulating sex steroid–binding protein has been studied by a number of investigators and remains controversial. Most of the studies conclude that there is no change[3-8] in sex steroid–binding protein concentrations; however, a decrease[9, 10] and an increase[11] in sex steroid–binding protein levels have also been reported. The present investigation was carried out to determine the effect of dexamethasone on plasma sex steroid–binding protein levels during the menstrual cycle of the rhesus macaque, with the use of a rigorous experimental design. We also observed the effect of dexamethasone on circulating levels of corticosteroid-binding globulin, cortisol, total and free testosterone, progesterone, and estradiol.

Material and methods

Animals. Four female rhesus macaques *(Macaca mulatta)* from the Oregon Regional Primate Research Center were used in this study. The animals weighed between 5.1 and 7.8 kg and had a history of regular menstrual cycles. They were housed in individual cages in light-controlled (lights on from 7:00 AM to 7:00 PM) and temperature-controlled (23° ± 2° C) quarters. The animals were given Purina Monkey Chow, and water was freely available to them. Examinations for menstrual bleeding were made daily, and the day of onset of menses was designated as day 1 of the cycle.

Treatment. The animals were studied during three

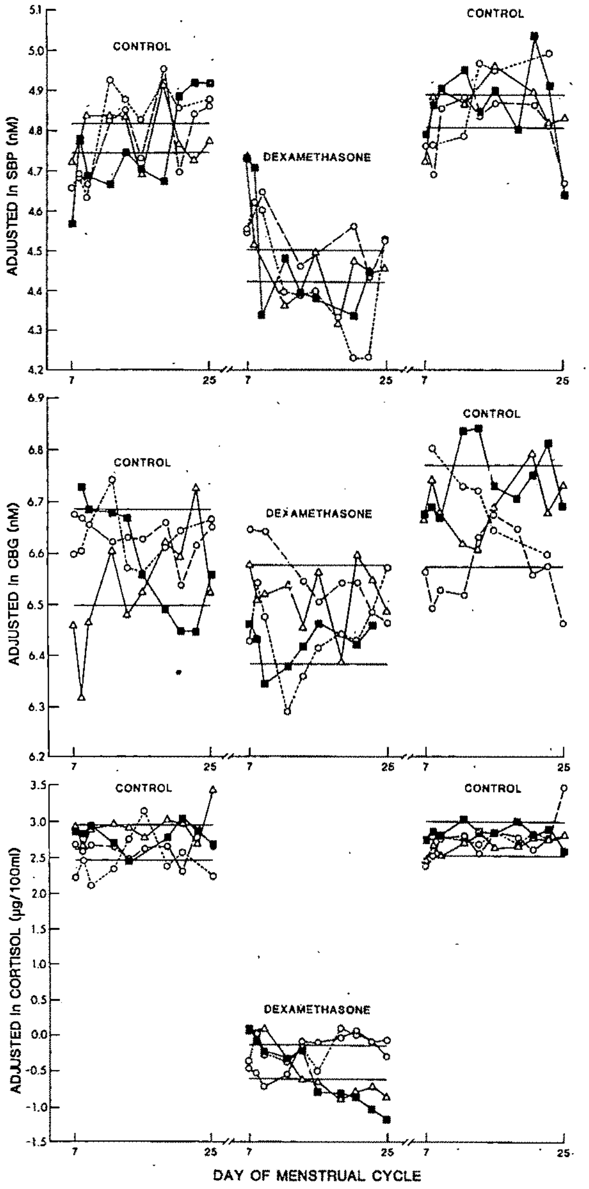

Fig. 2. Plasma sex steroid–binding protein *(SBP),* corticosteroid-binding globulin *(CBG),* and cortisol levels during the study periods in the three consecutive menstrual cycles of all four animals. The data were transformed to logarithms and adjusted for animal and cycle day differences. The 95% confidence limits of the menstrual cycle means are plotted for each compound.

consecutive menstrual cycles in which the treatment cycle was preceded and followed by a control cycle. One half milligram of dexamethasone (Decadron phosphate, Merck, Sharp & Dohme, West Point, Pennsylvania) was administered in the treatment cycle and 0.5 ml of normal saline solution was given in the control cycles by intramuscular injection at 8:00 AM and 8:00 PM daily for 21 consecutive days beginning at day 5 of each cycle.

Sampling. Blood samples (4 ml) were collected into heparinized syringes at 8:00 AM on days 5, 6, 7, 8, 9, 12, 14, 16, 19, 21, 23, and 25 of each cycle. The samples were obtained within 3 minutes of the initial approach by venipuncture in the animal's own cage. The blood was immediately centrifuged at 2000 rpm for 20 minutes at 4° C, and the plasma was frozen and stored at −20° C.

Assays. Plasma concentrations of sex steroid–binding protein, corticosteroid-binding globulin, cortisol, progesterone, estradiol, and testosterone were quantitated as follows: Sex steroid–binding protein and corticosteroid-binding globulin were measured by diethylaminoethyl cellulose filter paper assays as described previously.[12, 13] We have demonstrated previously that the sex steroid–binding protein filter assay correlates very highly with our enzyme-linked immunosorbent assay for sex steroid–binding protein and that the two assays may be used interchangeably.[14] Therefore, it is justified to use the filter assay to measure sex steroid–binding protein levels. Specificity studies by different investigators show insignificant binding of corticosteroids to sex steroid–binding protein[15, 16] and no binding of dexamethasone to corticosteroid-binding globulin.[17, 18] Progesterone, estradiol, and testosterone were measured at the Oregon Primate Research Center radioimmunoassay laboratory by assay methods described previously[19-21] and validated in that facility. These steroids were quantitated by radioimmunoassay following extraction of the compounds with diethyl ether and separation by LH-20 column chromatography. Cortisol was measured directly in diluted plasma as described by Walsh et al.[22]

Calculation of percentage of free testosterone. The percentage of free testosterone was calculated from equations derived from the mass action relationships for sex steroid–binding protein and albumin as described by Tabei et al.[23] Concentrations of sex steroid–binding protein, albumin, and total testosterone were obtained experimentally and used in the calculations. Albumin was quantitated colorimetrically and the concentration did not change significantly during the three study cycles of each animal. The equilibrium constants of association of testosterone to macaque sex steroid–binding protein ($K_{SBP} = 1.37 \times 10^8$ L/mol) and albumin ($K_{ALB} = 2.02 \times 10^4$ L/mol) were also used in the calculations. The absolute levels of free testosterone (picograms per milliliter) were calculated from the product of the total testosterone concentration and the percentage of the free hormone.

Statistical analysis. The data were analyzed by the Statistical Package for the Social Sciences[24] as a three-way repeated-measures factorial analysis of variance with unequal cell frequencies. The three factors were individual animal variation, day of cycle variation, and treatment effects. Sums of squares were calculated by the classic experimental design approach, and mean treatment effects were adjusted for animal and cycle

Table I. Effect of dexamethasone treatment as a percentage of control levels

Compound	*Mean plasma concentration*		% Difference	p†
	*Control**	*Treatment*		
Sex steroid–binding protein (nmol/L)	127	89	−30	<0.001
Testosterone (pg/ml)	450	290	−36	<0.05
Estradiol (pg/ml)	126	70	−45	<0.01
Corticosteroid-binding globulin (nmol/L)	766	662	−14	<0.05
Cortisol (μg/100 ml)	16.1	0.8	−95	<0.001
Progesterone (ng/ml)	2.32	2.15	− 7	>0.20

*The control represents the mean of values from the two untreated cycles.

†Significance of the t test for differences between dexamethasone-treated and untreated cycles in log transformed units.

day differences. The animal-by-treatment interaction served as the error term when a priori single degree of freedom contrasts of treatment effects were evaluated. Data were transferred to natural logarithms to achieve a higher multiple correlation coefficient.

Results

Plasma levels of sex steroid–binding protein, testosterone, estradiol, corticosteroid-binding globulin, cortisol, and progesterone throughout three consecutive menstrual cycles in a representative study animal are depicted in Fig. 1. Dexamethasone highly suppressed cortisol levels within 1 to 2 days of treatment. The levels returned to baseline values 5 to 6 days following discontinuation of treatment. Sex steroid–binding protein concentrations were decreased on the third day following the initiation of dexamethasone administration. Throughout the treatment cycle, sex steroid–binding protein levels were considerably lower and corticosteroid-binding globulin only slightly lower compared to the control cycles. All cycles were ovulatory, on the basis of elevated progesterone concentrations in the luteal phase. A midcycle peak of estradiol was observed in each cycle but appeared to be suppressed in the treatment cycle. Also testosterone levels were lower in the treatment cycle when compared to the control cycles.

Plasma sex steroid–binding protein, corticosteroid-binding globulin, and cortisol concentrations during the study periods in the three consecutive menstrual cycles of all four animals are shown in Fig. 2. The graphs show the observations adjusted for animal and cycle day differences with the 95% confidence limits around the mean value of each compound in all the cycles. Data from the first two days of dexamethasone treatment were eliminated in all animals because cortisol levels were not yet maximally suppressed in the treatment cycle. The results show that dexamethasone completely suppressed baseline cortisol concentrations which returned to the former levels in the subsequent control cycle. Levels of sex steroid–binding protein were considerably lower in the treatment cycle compared to the control cycles. Levels of corticosteroid-binding globulin in the dexamethasone cycle were only slightly lower than the control values.

The effect of dexamethasone treatment as a percentage of control plasma sex steroid–binding protein, corticosteroid-binding globulin, and steroid hormone levels is shown in Table I. Dexamethasone suppressed cortisol levels to 5% of the control values. The sex steroid–binding protein level decreased by 30% and corticosteroid-binding globulin by 14%. We also observed sizable decreases in the sex steroids, ranging from 7% to 45%. The differences between mean control and treatment cycles in log transformed units are significant for all the compounds except progesterone (Table I).

The adjusted means and standard errors of plasma sex steroid–binding protein, total testosterone, and free testosterone concentrations, as well as the percentage of free testosterone in the three consecutive study cycles, are shown in Table II. As indicated before (Table I), dexamethasone significantly lowered sex steroid–binding protein and total testosterone levels from control values. During dexamethasone treatment cycles, the mean percentage of free testosterone was significantly higher than the corresponding mean control values. However, the mean free testosterone concentrations in the dexamethasone-treated and untreated cycles did not differ significantly.

Comment

In the present study we attempted to gain an understanding of the observations in primates and women that the metabolic clearance rates of estradiol and testosterone are influenced by pharmacologic doses of dexamethasone. We explored the possibility that these findings may be explained by a mechanism of altered plasma sex steroid–binding protein concentration rather than a change in the pathways of steroid biosynthesis and/or metabolism. The results of this study provide the first direct evidence that dexamethasone lowers plasma levels of sex steroid–binding protein in a primate species. Dexamethasone lowered sex

Table II. Comparisons of plasma levels of sex steroid–binding protein, total and free testosterone in transformed units, and percentage of free testosterone in dexamethasone-treated and control cycles

	Control		*Treatment*	
	Adjusted mean ± SE	*Sample size*	*Adjusted mean ± SE*	*Sample size*
Log normal sex steroid–binding protein	4.81 ± 0.013	70	4.46* ± 0.018	33
Log normal total testosterone	5.99 ± 0.105	75	5.51† ± 0.144	39
Log normal free testosterone	2.73 ± 0.063	66	2.45 ± 0.079	33
% free testosterone	3.84 ± 0.093	66	4.77* ± 0.131	33

*Significantly different from the mean of the two control cycles ($p<0.001$).

†Significantly different from the mean of the two control cycles ($p<0.05$).

steroid–binding protein levels from control values by 30%. This decrease was consistent as evidenced by the high t statistic for sex steroid–binding protein (16.325).

As expected, the percentage of free testosterone increased in the treatment cycle of each animal (Table II) because of the reduced levels of sex steroid–binding protein. The calculated percentage of free testosterone is dependent on the plasma levels of sex steroid–binding protein, albumin, and total testosterone. Under the present experimental conditions, the concentration of albumin did not change in the control and treatment cycles of each animal. Also, mathematical modeling[23] shows that the percentage of free testosterone is nearly independent of total testosterone concentrations over the normal range of values. Therefore, the major controlling factor which determines the percentage of free testosterone is sex steroid–binding protein.

The absolute concentration of free testosterone did not change significantly during the treatment cycles, despite lowered peripheral levels of total testosterone during that period (Table II). Dexamethasone suppresses the normal secretion of adrenal testosterone as well as androstenedione.[25] In approximate amounts, the adrenal gland directly contributes 25% to the circulating levels of testosterone, and another 25% of this androgen arises from peripheral conversion of adrenal androstenedione; the remaining 50% of circulating testosterone originates from the ovary.[26, 27] The degree of adrenal androgen suppression by dexamethasone is related to both the dosage and the duration of administration of the drug. In the present study, dexamethasone decreased testosterone levels by 36% from control levels (Table I).

In their study of the effect of dexamethasone (2 mg per day) on plasma sex steroid–binding protein and free androgen levels during three periods (early follicular, midcycle, and midluteal) of the menstrual cycle in normal women, Kim et al.[3] concluded that sex steroid–binding protein levels were not affected by dexamethasone because mean plasma levels were similar during treated and untreated cycles. However, this conclusion is based on blood samples from only two women who were studied sequentially in normal and treatment cycles. Moreover, one woman demonstrated a 30% to 40% decrease in sex steroid–binding protein levels during the treatment cycle, whereas the sex steroid–binding protein concentrations were unaltered in the second subject. A third subject was studied only during the treatment cycle and had sex steroid–binding protein levels approximately twofold higher than the corresponding values observed in the other two subjects (in either treatment or control cycles), thereby resulting in biased elevated mean sex steroid–binding protein values after dexamethasone administration. In the same study, Kim et al.[3] found that the plasma levels of total and free testosterone during dexamethasone suppression were significantly lower than those found during normal cycles. They attributed the decreased values to reduction of adrenal androgen production by dexamethasone, assuming that the glucocorticoid had no significant effect on normal ovarian function.

Several other studies have examined the effect of dexamethasone on plasma sex steroid–binding protein and total and free testosterone levels in women with idiopathic hirsutism and hirsutism associated with polycystic ovarian syndrome. In hirsutism, concentrations of total and free testosterone are usually elevated. Long-term suppression of elevated androgens by dexamethasone administration has been recommended by some investigators as the primary form of therapy in patients with hirsutism.[27] However, there is disagreement regarding the efficacy of this type of treatment on circulating androgens. Some investigators[4, 6, 9] have reported a significant decrease in total testosterone as well as free and/or unbound (not sex steroid–binding protein bound) testosterone levels in patients with idiopathic hirsutism or polycystic ovarian syndrome following dexamethasone administration (0.5 to 2 mg per day). On the other hand, Lachelin et al.[8] took into consideration the glucocorticoid effect on circadian fluctuation of hormones and found only a 15% decrease in total testosterone levels in four patients with polycystic ovarian syndrome, in comparison to a 46% decrease in normal women, after dexamethasone administration (1 mg per day) for 1 month; free testosterone was not measured. Levels of sex steroid–binding protein or testosterone bound to sex steroid–binding protein were also measured in the same studies,[4, 6, 8, 9] and the data are conflicting. Wu[9] found that the mean percentage of testosterone bound to sex steroid–binding protein was decreased significantly from pretreatment values and suggested that dexamethasone (2 mg per day) may deplete sex steroid–binding protein levels. In the other studies, the investigators[4, 6, 8] reported no change in sex steroid–binding protein levels following dexamethasone administration (0.5 to 1 mg per day) for 2 weeks to 1 month. Therefore, only the data from Wu[9] are consistent with our findings of suppressed sex steroid–binding protein levels in response to dexamethasone. The reason(s) for the unaltered sex steroid–binding protein levels in the other studies[4, 6, 8] are unknown, although the dose and schedule of dexamethasone treatment and sampling frequency may be significant factors. In the studies quoted above, comparisons were made between sex steroid–binding protein levels measured in a very limited number (usually one) of pretreatment and posttreatment samples. An attempt to compare sex steroid–binding protein levels in women, consistently over a wide range of days as we have done in the rhesus, would be worthwhile.

In our study, dexamethasone also lowered plasma corticosteroid-binding globulin and estradiol levels from control values but had no significant effect on progesterone concentrations (Table I). The decrease (14%) in corticosteroid-binding globulin in response to dexamethasone was lower and more variable than the effect on sex steroid–binding protein. The *t* statistic for corticosteroid-binding globulin was low (3.074), reflecting the large variability of this binding protein between animals and between cycles (Fig. 2). Dexamethasone apparently had no effect on ovulation as judged by the luteal phase increases of progesterone concentrations. Similar data have been reported for progesterone in women.[25] Although dexamethasone decreased estradiol concentrations by 45% from control values, it is not likely that this decrease resulted in lowered sex steroid–binding protein levels since estradiol does not stimulate sex steroid–binding protein production in the adult female rhesus macaque.[28, 29] Dexamethasone probably lowers sex steroid–binding protein levels by a direct effect on hepatic steroid biosynthesis as opposed to a secondary effect of lowered estradiol concentrations. In the human, there is no significant effect of dexamethasone on estradiol concentrations.[25] The possibility that the decrease in estradiol from control levels in the present study is due to infrequent sampling cannot be excluded, since blood samples were obtained at 2-day intervals around the time of the midcycle estradiol peak.

There is evidence in rhesus macaques and in women that the administration of pharmacologic doses of dexamethasone or adrenocorticotropic hormone results in an increase in the metabolic clearance rate of estradiol within 1 to 3 days after treatment is initiated.[1, 2] It has previously been calculated that 80% of the total metabolic clearance rate of estradiol in the adult female rhesus macaque may be accounted for by splanchnic estradiol uptake alone.[30] Thus, an increase in hepatic blood flow could explain the increase in the metabolic clearance rate of estradiol, but it should also result in an increase in the metabolic clearance rate of estrone. Since the latter was not observed in the study by Longcope et al.,[1] it is unlikely that increased blood flow alone is the cause for the elevated metabolic clearance rate of estradiol after dexamethasone treatment. Alternatively, dexamethasone may increase tissue metabolism of estradiol or, as we have observed, decrease the size of the sex steroid–binding protein pool. We have previously reported in the female rhesus macaque that the metabolic clearance rate of testosterone (liters per day per kilogram) was depressed when plasma sex steroid–binding protein levels were acutely elevated by infusion of purified sex steroid–binding protein and increased after the administration of a specific antiserum against sex steroid–binding protein.[31] There was approximately a three-fold increase in the metabolic clearance rate of testosterone when the sex steroid–binding protein level fell to 96% of control values. It is improbable that a 30% decrease in sex steroid–binding protein levels, as observed in response to dexamethasone in the present study, would be the sole explanation of the fivefold to sixfold increase in the metabolic clearance rate of estradiol observed by Longcope et al.[1] because the binding affinity of estradiol for sex steroid–binding protein is significantly lower than that of testosterone. Therefore, it is likely that dexamethasone must also increase the tissue metabolism of estradiol.

The results of the present study show that dexamethasone significantly lowers plasma sex steroid–binding protein levels and increases the percentage of free testosterone in the rhesus macaque. That the concentration of free testosterone did not change significantly suggests that the metabolic clearance rate of this hormone was elevated from basal values while the production rate was decreased by dexamethasone treatment. In order to determine conclusively whether administration of pharmacologic amounts of dexamethasone results in a marked increase in the metabolic clearance rate of testosterone in primates, studies in which the metabolic clearance rate of this hormone and plasma concentrations of sex steroid–binding protein and free testosterone are measured simultaneously must be carried out.

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Frequency of diabetes mellitus in mothers of probands with gestational diabetes: Possible maternal influence on the predisposition to gestational diabetes

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Interviews for genetic histories were conducted prior to delivery in 166 pregnant diabetic probands and 83 control gravidas with normal carbohydrate metabolism throughout gestation. A significant association was observed between parental diabetic phenotypes and type of diabetes in the probands ($\chi^2_{(12)}$) = 32.413; $p < 0.001$). In particular a higher than expected number of mothers with diabetes was encountered in 91 probands with gestational diabetes mellitus. The findings are examined in relationship to the hypothesis that vulnerability to gestational diabetes may be increased by exposure to an abnormal environment during intrauterine development. (AM J OBSTET GYNECOL 1985;151:471-5.)

Key words: Gestational diabetes, familial, maternal influences

The proposition that altered maternal metabolism may exert long-term impact on offspring by conditioning the metabolic mixtures available to certain terminally differentiated, poorly replicating cells during their intrauterine development ("fuel-mediated teratogenesis"[1]) has formed the basis for the prospective studies of the Northwestern University Diabetes in Pregnancy Center. We have postulated that perturbations in the intrauterine environment may modify the phenotype predicted on the basis of genetic endowment.[1, 2] Accordingly, the neurological, psychological, intellectual, anthropometric, and metabolic characteristics of the progeny of diabetic mothers are being correlated with the metabolic status of the mother throughout the index pregnancy.

That β-cells of the fetal pancreas might be particularly vulnerable to long-range modification via the intrauterine environment has been suggested previously.[1, 2] β-Cells not only replicate poorly[3, 4] but also are notoriously subject to proliferation and premature secretory maturation in utero as result of increased stimulation with nutrient secretagogues in maternal diabetes.[2, 5-7] Excessive nutrient challenges during β-cell functional maturation could predispose to premature senescence or functional deterioration during later life, with overt diabetes becoming manifest.[1, 2] While examining this possibility prospectively, we have simultaneously attempted to secure retrospective insights by evaluating the frequency of diabetes among the parents of our probands. This report summarizes some of our emerging evidence suggesting that gravidas with gestational diabetes mellitus are more likely to have mothers with diabetes than are pregnant women with pregestational insulin-dependent diabetes mellitus or normal glucose regulation. The provocative implications of these preliminary observations prompt publication to stimulate investigations by others.

Material and methods

Sample. All women attending the Obstetric Clinics of Prentice Women's Hospital are screened for gestational diabetes mellitus at 24 to 28 weeks of gestation on the basis of plasma glucose values (≥140 mg/dl) secured 1 hour after oral challenge with 50 gm of glucose. Definitive diagnosis of gestational diabetes mellitus is established according to the criteria of O'Sullivan and Mahan[8] after an oral glucose tolerance test with 100 gm of glucose. We have been distinguishing between patients with gestational diabetes mellitus with normal values for fasting plasma glucose (<105 mg/dl)[8] and those with increased values for fasting plasma glucose (≥105 mg/dl)[9] on the assumption that "underutilization" constitutes the principal metabolic disturbance in those with normal values, whereas those with increased values are experiencing varying degrees of "overproduction" in addition.[2] Similar subdivisions of gestational diabetes mellitus on the basis of fasting plasma glucose levels were also advocated at the First International Workshop on Gestational Diabetes[10] and

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Table I. Status of parents of diabetic probands and control subjects

	Normal father, normal mother		*Normal father, diabetic mother*		*Diabetic father, normal mother*		*Diabetic father, diabetic mother*		
Status of proband	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Total*
Gestational diabetes mellitus, no prior history of diabetes	29	54	19	35	4	7	2	4	54
Gestational diabetes mellitus, prior history of diabetes	22	59	11	30	4	11	0	0	37
Class B2, modified White Class B	15	88	2	12	0	0	0	0	17
White Class, C, D, or F	44	76	7	12	5	9	2	3	58
Control subjects	72	87	4	5	5	6	2	2	83
Total	182		43		18		6		249

$\chi^2_{(12)} = 32.413$; $p < 0.001$.

by the National Diabetes Data Group.[11] In accordance with their recommendations, we have confined the diagnosis of gestational diabetes mellitus to "glucose intolerance with onset or recognition during pregnancy." However, for optimal clarity, we distinguish between patients with gestational diabetes mellitus in whom the diagnosis is made for the first time during the index pregnancy (that is, no prior history) and those in whom glucose intolerance has been previously diagnosed, usually during an earlier pregnancy (that is, prior history) although no hypoglycemic therapy is being administered. We designate as Class B2 (modified White Class B) those patients with pregestational diabetes mellitus who conform to the criteria for White Class B and who are already receiving insulin therapy at the initial antepartum visit.[9] In many instances, definitive classification of the patients in this category as type I or type II diabetes mellitus (that is, insulin-dependent diabetes mellitus or non–insulin-dependent diabetes mellitus) has been difficult because of the growing recognition that type I, like type II, diabetes mellitus may be a slowly evolving disorder.[12, 13] On the other hand, we have retained the traditional White classifications[14] (Classes C, D, and F) for the remainder of our pregestational diabetic patients since they almost invariably conform to the type I designation. As part of the Diabetes in Pregnancy program, we have also recruited a limited number of control gravidas in whom normal carbohydrate tolerance was documented by screening and a subsequent oral glucose tolerance test.

From all of these subjects we obtained detailed genetic histories through interviews that were usually conducted prior to delivery; however, a few were obtained after delivery. Interviews of 193 diabetic women and 36 control subjects were conducted on the basis of a standard format by the investigators or by interviewers trained and supervised by the investigators. If family members were said to have diabetes, further details were sought concerning age of onset, disease progression, and type of treatment (diet only, oral hypoglycemics, insulin). Parents were considered to have diabetes only if the proband could attest to their receiving insulin or oral hypoglycemic medications. Families were excluded prior to genetic analysis if probands were adopted, if no information concerning the health of their parents was available, or if the diabetic status of the parents was uncertain. Twenty-nine probands (26 diabetic subjects, three control subjects) were excluded for these reasons. The remaining 167 diabetic patients were classified according to clinical status during their most recent pregnancy.

Within the stated framework, our present report is based on the findings in 54 patients with gestational diabetes mellitus with no prior history of glucose intolerance (fasting plasma glucose level <105 mg/dl in 32 and ≥105 mg/dl in 22), 37 patients with gestational diabetes mellitus with a prior history (fasting plasma glucose level <105 mg/dl in six and ≥105 mg/dl in 31), 17 patients in Class B2 (modified White Class B), 58 patients in White Class C, D, or F; and 33 control subjects. One patient in the group with gestational diabetes mellitus was excluded because no information was available on her prior history. Because our initial control sample was relatively small, we subsequently chose a second control group of 50 gravidas from the same patient population to verify that our original control was representative. Women in the second control group included all gravidas appearing sequentially in the Prentice Women's Hospital Obstetric Clinics during a specific time interval in 1983. In all cases, normal carbohydrate status was confirmed during the ongoing pregnancy. These women were interviewed for their family history in the same fashion as the initial concurrent control group and by the same interviewers.

Ethnic classification was made on the basis of grandparental origin: white, black, Hispanic, etc. Because of the small numbers involved in some ethnic groups, all Spanish-speaking groups were grouped as "Hispanic." Persons of mixed origins were classified as "other" as were persons neither white, black, nor "Hispanic."

The relationship between clinical status of the probands and that of their parents is summarized in Table I. χ^2 analysis was performed to determine whether parental mating type distributions were the same among the classes defined by the proband's clinical status. The contribution of the various cells (cross classifications in Table I) to the overall χ^2 was noted to determine relative importance of deviations.

Results

Distribution of parental mating types is shown in Table I. Results in the two sets of controls were not significantly different; thus data were pooled for the final analysis. A significant association between parental diabetes and the type of diabetes shown by the probands was observed ($\chi^2_{(12)} = 32.413$; $p < 0.001$). In particular there exists an excess over the expected numbers of mothers with diabetes among the 91 probands with gestational diabetes mellitus.* The contribution to χ^2 is highest in the 54 patients from this population with no prior history of diabetes. This pattern was not observed for the 58 pregestational diabetics in White Classes C, D, and F or in the 83 gravidas with normal carbohydrate metabolism throughout gestation (controls, Table I). Little can be inferred from Class B2 because of small numbers and the uncertainties about the pregestational categorization as type I or type II diabetes mellitus.

Some differences in the ethnic composition existed between the probands of the various diabetic groups and the control subjects. However, the increased frequency of a parental pattern consisting of a normal father and a diabetic mother persisted in the gestational diabetes mellitus groups even when ethnic groups were examined separately, although the numbers in the individual categories were small after such subdivision.

For the present study it proved to be impossible to categorize with precision the type of diabetes in the parents or the ages of the parents at the onset of the diabetes. Retrospective attempts to assess status of maternal carbohydrate metabolism during the gestation of the proband are not likely to be successful because few medical records relating to such an interval 20 to 30 years ago have been retained; standard clinical practice at that time did not include screening for gestational diabetes, and proband histories were not deemed reliable in this regard.

Comment

Genetic histories secured from 166 consecutive pregnant diabetic women in our Diabetes in Pregnancy program have indicated that patients with gestational diabetes mellitus are relatively more likely to have a mother with diabetes than gravidas with normal carbohydrate metabolism (control subjects) and other classes of diabetic probands. We encountered a history of diabetes mellitus in the mothers of approximately one third of all of our patients with gestational diabetes mellitus, with a slightly higher frequency in those with no prior history of any carbohydrate intolerance. Histories of pregestational diabetic patients (White Classes C, D, F) do not show this pattern. In this regard, Tattersall and Fajans[15] have previously noted that the frequency of parental diabetes is much lower in juvenile-onset diabetes than in the maturity-onset type of diabetes of young people (although their report did not include a breakdown of the incidence of diabetes in the fathers vis-à-vis the mothers).

There are several possible explanations for our findings. First, the observations could be merely fortuitous and perhaps not verifiable in a larger series. Second, our study, as with all questionnaire surveys, has inherent limitations. Recall of parental health is not always reliable. Even so, it is not clear why such recollection should be different in patients with gestational diabetes mellitus than in gravidas with type I diabetes mellitus or normal carbohydrate metabolism. Third, there may be a higher prevalence of diabetic women resulting from either a sex-limited diabetic gene or earlier age of onset in females predisposed to diabetes. One would have to invoke this as a peculiarity of the mothers of our gestational diabetes mellitus population and, indeed, non–insulin-dependent diabetes is found more frequently in women than in men in this age group.[16] However, it is uncertain whether this is truly a sex-limited phenomenon or merely a reflection of the greater prevalence of obesity in middle-aged women than in middle-aged men. The relationship to parental gender appears to be somewhat different in insulin-dependent diabetes. Wagener et al.[17] observed a significantly greater incidence of diabetes in the fathers than in the mothers of patients with newly discovered type I diabetes. Fourth, it might reflect, at least in part, a difference in the ages of our various groups. Indeed, our gestational diabetic patients were slightly older (mean ages = 31.6 years, SE = 0.8 for patients with gestational diabetes mellitus with no prior history and 33.4 years, SE = 1.0 for those with prior history) than our pregestational diabetic patients of White Classes C, D, and F (mean age = 25.6 years, SE = 0.6) or control gravidas (mean age = 25.3 years, SE = 0.6), so that the mothers of the patients with gestational diabetes mellitus potentially had more years in which to manifest diabetes. In practice, however, this does not seem to explain our observations, because the differences remain when we restrict our scrutiny to women under the age of 30 years (39% of the 28 gestational diabetes mellitus probands <30 years of age have mothers with diabetes compared to the 6% of the 66 control subjects

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*The highly significant association persists after regrouping parental phenotypes by status of the mother only (normal versus diabetic).

<30 years of age). Thus, it seems that other determinants should be invoked.

Within the context of our data, a fifth speculative explanation merits consideration. Conceivably the greater frequency of maternal than paternal diabetes in our gravidas with gestational diabetes mellitus may be relevant to the actual pathogenesis of gestational diabetes mellitus. We propose that many of these gravidas may have been exposed to an abnormal environment during their own intrauterine development by virtue of their mother's diabetes and that their β-cells may have been subjected to inordinate challenges during late gestation. As a consequence, an increased vulnerability of their β-cells may have become established in utero on nongenetic grounds and subsequently unmasked as gestational diabetes mellitus in the course of the normal diabetogenic challenges of pregnancy. The failure to observe this in our Class C, D, and F pregnant diabetic patients may be due to the operation of other factors in insulin-dependent diabetics that obscure and/or override the intrauterine contributions of maternal metabolism. Admittedly we cannot be certain that maternal carbohydrate intolerance was present at the time that our probands with gestational diabetes mellitus were developing in utero. Even so, it does not seem to be an unreasonable likelihood, because the diabetes in most of their mothers appeared to be of a non–insulin-dependent variety, at least by history, and hence may already have been operative on an intermittent basis during the gestation of the probands.

Some published reports are consistent with the above hypothesis. Aerts and Van Assche[18] have described inadequate pancreatic β-cell responses during pregnancy in the offspring of genetically normal rats whose mothers had been rendered diabetic by the administration of streptozotocin. Gestational β-cell limitations persisted in the second as well as third generation of such rats and prompted Aerts and Van Assche to conjecture that gestational diabetes could represent an acquired disorder.[18] Similar preliminary observations have been made by Pettit et al.[19, 20] in their study of Pima Indians, a group in whom the incidence of non–insulin-dependent diabetes mellitus is inordinately great. They have observed significantly more obesity[19] and diabetes[20] in the offspring (up to the age of 19 years) of Pima mothers who were diabetic during the index pregnancy than in those whose mothers remained nondiabetic or did not become diabetic until after the pregnancy. Although the data on diabetic fathers have not yet been published, Pettitt et al.[19, 20] have suggested that the differences in offspring may be due to differences in the maternal glucose regulation during pregnancy rather than genetic factors and thereby consistent with the proposal that the intrauterine environment may exert long-range effects upon certain poorly replicating, terminally differentiated cells such as adipocytes or pancreatic β-cells.[1, 2]

Clearly the proposition that alterations in fuel metabolism during late intrauterine development may predispose to gestational diabetes mellitus during adult life must still be viewed as a hypothesis. Nonetheless, our data emphasize the desirability of gathering additional observations along these lines in larger series as well as in different ethnic and racial groups. The information not only should prove useful in elucidating the environmental factors that influence the expression of a non–insulin-dependent type of diabetes but also might be of practical value. Confirmation that intrauterine events contribute to gestational diabetes in adult life would justify further aggressive attempts to normalize even the mildest disturbances of maternal metabolism during pregnancy. Such endeavors would provide a relatively simple and cost-effective preventative approach to the public health problem that the escalating incidence of non–insulin-dependent diabetes mellitus represents.

We are indebted to all of the professional and allied health members of the Northwestern University Diabetes in Pregnancy Center for their enthusiastic and spirited participation in the care of these patients. We are particularly grateful to Dr. Richard L. Phelps and Dr. Boyd E. Metzger for their contributions to the medical management, Dr. Richard Depp and Dr. Sharon Dooley for the obstetric supervision, and Dr. John Boehm and Dr. Edward Ogata for the neonatal care.

Addendum

Since the submission of this manuscript, a retrospective analysis of patients seen at the Joslin Clinic between 1928 and 1939 has indicated that type I insulin-dependent diabetes mellitus developed with significantly less frequency in the surviving progeny of mothers with insulin-dependent diabetes mellitus than in progeny of fathers.[21] The findings in type I diabetes mellitus are not necessarily at variance with our observations in gestational diabetes mellitus. They highlight the fundamental differences between pregestational type I diabetes mellitus and the type of diabetes in which metabolic disturbances in the mother and attendant effects on intrauterine development[22] are confined in large measure to the latter half of gestation (gestational diabetes mellitus).

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Catecholamine responses in fetal lambs subjected to hemorrhage

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We monitored plasma epinephrine and norepinephrine responses to hemorrhage of 20% of estimated blood volume in 11 chronically instrumented, unanesthetized fetal lambs. In addition, we performed control experiments—blood sampling but no hemorrhage—in five fetuses. Arterial blood gases, pH, mean arterial pressures, heart rates, and plasma norepinephrine and epinephrine levels were similar in both groups in the resting state. Arterial blood gases and pH did not change significantly during the experimental period in either group. Mean arterial pressure, heart rate, and plasma norepinephrine and epinephrine levels did not change in the control group during the experimental period. Hemorrhage was associated with a significant decrease in fetal mean arterial pressure, 39.8 ± 1.2 to 29.6 ± 2.1 mm Hg, and heart rate, 186 ± 8 to 146 ± 6 bpm ($p < 0.01$ in both cases). There was a significant increase in plasma norepinephrine, 664.9 ± 91.6 to 1384.8 ± 216.7 pg/ml ($p < 0.02$) and epinephrine, 224.6 ± 43.6 to 681.7 ± 199.0 pg/ml ($p < 0.01$) with hemorrhage. These results demonstrate significant catecholamine responses to hypovolemia in the fetal lamb. (Am J Obstet Gynecol 1985;151:475-8.)

Key words: Fetus, hemorrhage, catecholamines

Hemorrhage is known to induce a number of neural and endocrine reflexes which work to maintain adequate perfusion pressure and to restore blood volume. The endocrine responses to acute hypovolemia have been well documented in the adult and include increases in catecholamines, vasopressin, renin, adrenocorticotropic hormone, and corticosteroids.[1] However, limited data describing fetal endocrine responses to

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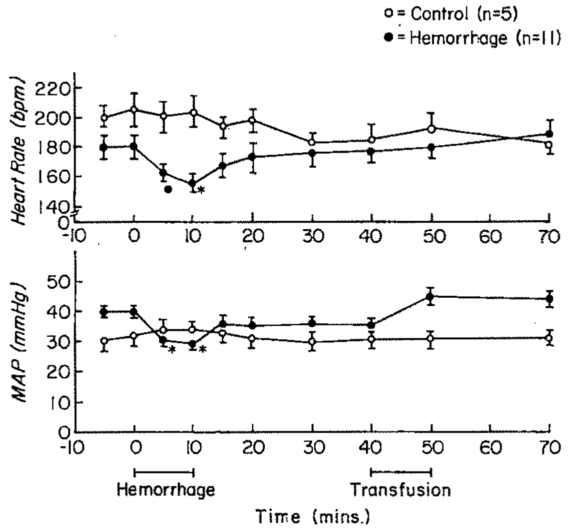
Table I. Arterial blood gases, pH, and hematocrits data for hemorrhage and control groups

	Before hemorrhage	*After hemorrhage*	*Before transfusion*	*After transfusion*
Hemorrhaged group (n = 11)				
pH	7.44 ± 0.04	7.41 ± 0.05	7.40 ± 0.06	7.42 ± 0.03
Pa_{O_2} (mm Hg)	22.5 ± 5.1	21.1 ± 4.0	21.4 ± 3.5	18.1 ± 3.0
Pa_{CO_2} (mm Hg)	34.3 ± 7.9	35.1 ± 9.3	33.3 ± 8.6	33.2 ± 8.8
Hematocrit (%)	29.6 ± 2.4	29.8 ± 3.9	29.9 ± 2.9	30.3 ± 2.8
Control group (n = 5)				
pH	7.39 ± 0.05			7.38 ± 0.05
Pa_{O_2} (mm Hg)	23.0 ± 3.2			23.4 ± 1.8
Pa_{CO_2} (mm Hg)	32.8 ± 6.1			32.1 ± 7.0
Hematocrit (%)	30.1 ± 1.2			29.2 ± 2.1



*p<0.01 when compared to respective resting values

Fig. 1. Heart rates and mean arterial pressures *(MAP)* in the hemorrhaged and control groups (mean ± SEM).

acute hemorrhage are available. In particular, it is not known whether volume depletion increases sympathoadrenal outflow, as reflected by circulating catecholamines, in the fetus as it does in the adult. Therefore the present study was designed to investigate the fetal sympathoadrenal response to acute fetal hypovolemia in chronically instrumented sheep.

Material and methods

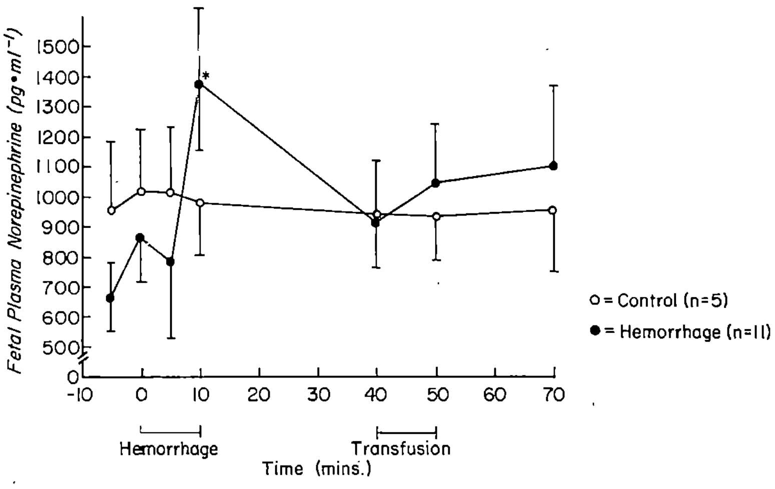
Ewes with time-dated gestations from 119 to 132 days (0.82 to 0.91 gestation) were housed within the research vivarium with access to food and water. Following a 4-day equilibration period the animals (n = 14) were taken to surgery where the ewe and fetus (two sets of twins) were chronically instrumented with indwelling catheters placed in the respective aorta, inferior vena cava, and amniotic cavity as previously described.[2] The animals were allowed a 3- to 4-day recuperation period following surgery and prior to experimentation. Maternal and fetal arterial blood gases, pH, and hematocrit were monitored during this period to assure stability of the preparation. Intra-amniotic kanamycin, 250 mg, was administered daily for 5 postoperative days and every other day thereafter. Minimal volumes of isotonic heparinized saline solution were used to flush the catheters and maintain patency. No experimental studies were performed during the recovery period. All studies were conducted between 12 noon and 5 PM as the ewe stood in a metabolic stall within an isolation room with minimal environmental stimulation.

The experimental protocol was as follows: Animals were assigned to the hemorrhage group (n = 10, one set of twins) or the control population (n = 4, one set of twins). Following a 30-minute equilibration period within the isolation chamber we removed 20% of estimated fetal blood volume calculated, from the fetal weight estimated by gestational age as previously described,[2] at 2% per minute from the hemorrhage group. The blood was obtained aseptically in heparinized syringes and stored in a 37° C water bath. After 30 minutes the shed blood was returned to the fetus over a 10-minute period.

Arterial blood samples for blood gases, pH, hematocrit, and plasma catecholamine levels (0.5 ml) were obtained prior to and at the end of the hemorrhage as well as at the beginning and termination of the reinfusion. The control animals were treated identically except that no hemorrhage was performed.

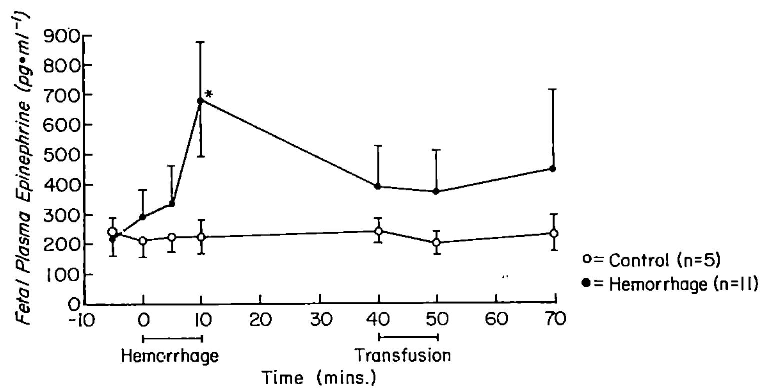
Maternal and fetal arterial pressures, heart rate, and amniotic cavity pressures were continuously monitored throughout the experiments via Statham P23Db transducers and recorded on a Beckman Dynograph recorder. Maternal and fetal arterial pressures were reported as mean arterial pressure calculated by the following formula: ⅓ of pulse pressure + diastolic pressure = mean arterial pressure. Fetal arterial pressures were corrected for intra-amniotic pressure.

Blood gases and pH levels were measured on a Radiometer BMS3 Mk2 blood system. Plasma catechol-



*p<0.02 when compared to resting values

Fig. 2. Fetal plasma norepinephrine concentrations in the hemorrhaged and control groups (mean ± SEM).



*p<0.01 when compared to resting values

Fig. 3. Fetal plasma epinephrine concentrations in the hemorrhaged and control groups (mean ± SEM).

amine concentrations were measured by a modified radioenzymatic technique of Peuler and Johnson[3] as previously described.[4] All samples from a particular experiment were analyzed in the same assay. Assay sensitivity was 2 to 3 pg/50 μl for norepinephrine and epinephrine. The interassay coefficients of variation were 7.1% and 9.0% for norepinephrine and epinephrine, respectively. The intra-assay coefficients of variation were 3.7 for norepinephrine and 4.4% for epinephrine. All values are reported in picograms per milliliter of plasma.

We analyzed the catecholamine and cardiovascular responses across time with the use of one-way analysis of variance, corrected for repeated measures. The initial control values in each group were compared with the *t* test. Additionally, because of the inhomogeneity of variance in the plasma catecholamine levels, the values were log transformed prior to analysis. Linear regression analysis was utilized in correlation analyses.

Results

Fetal arterial blood gases and pH data are summarized in Table I. There were no significant differences between the control or hemorrhage group pH, Pa_{O_2}, Pa_{CO_2}, and hematocrit values obtained prior to manipulation. Additionally, no significant differences were found in these parameters in either group over the experimental period. All fetal preparations appeared well oxygenated and without evidence of hypercapnia or acidosis.

Fig. 1 summarizes the fetal mean arterial pressure and heart rate data. There was no difference in resting mean arterial pressure between the control and hemorrhage group. There was a fall in mean arterial pressure following hemorrhage, 39.8 ± 1.2 to 29.6 ± 2.1 mm Hg ($p < 0.01$). There was no change in mean arterial pressure over the experimental period in the control population. There was no difference in resting heart rate between the hemorrhage and control group: 186 ± 8 and 190 ± 6 bpm, respectively (mean ± SEM). Following the hemorrhage, the fetal heart rate fell to 146 ± 6 bpm ($p < 0.01$). The heart rate did not change significantly over the experimental period in the control population.

The fetal norepinephrine and epinephrine concentrations are summarized in Figs. 2 and 3. There were no differences in resting norepinephrine and epinephrine concentrations between the two populations. There was a significant increase in circulating norepinephrine following the 20% hemorrhage: 664.9 ± 91.6 to 1384.8 ± 216.7 pg/ml of plasma ($p < 0.02$). Similarly, plasma epinephrine rose from a resting level of 284.6 ± 43.6 to 681.7 ± 199.0 pg/ml following the hemorrhage ($p < 0.01$). There was no significant change in norepinephrine and epinephrine in the control group over the experimental period. The resting concentrations of norepinephrine were greater than the resting concentrations of epinephrine ($p < 0.01$).

Comment

In adult animals hemorrhage is associated with an increase in plasma norepinephrine and epinephrine concentrations that result, at least partially, from an increase in the secretion of these catecholamines from the adrenal medulla.[5-8] In the present experiments we demonstrated that the fetus also shows a significant sympathoadrenal response to hemorrhage. Volume depletion of 20% estimated blood volume produced a 2.1-fold increase in fetal plasma norepinephrine concentrations and a 3.0-fold increase in circulating epinephrine levels. Clearly these increases were not caused by changes in blood gases or pH nor were they a consequence of the sampling procedure itself. The actual stimulus to increased sympathoadrenal activity following hemorrhage in the fetus is probably related to the fall in fetal arterial pressure produced by the hemorrhage. In adult animals reductions in blood pressure following hemorrhage are inversely related to adrenal medullary secretion rates of norepinephrine and epinephrine.[7] The receptors mediating the catechol responses may be carotid sinus or cardiopulmonary mechanoreceptors. Although there is some disagreement, both have been implicated in the control catecholamine secretion in adult animals.[3, 9]

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An examination of vascular reactivity during pregnancy in the chronic ewe model

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The hind limb vascular responses to angiotensin II and phenylephrine, an α-adrenergic agonist, were determined in chronically instrumented pregnant (n = 7) and castrated (n = 6) ewes. On any one day, five doses of one or the other drug were administered directly into the arterial femoral circulation while the perfusion pressure and external iliac blood flow were monitored. The steady-state resistance change (baseline resistance − the resistance calculated during the 3 to 5 minutes of drug infusion) showed a reduction in vascular response to angiotensin II in the pregnant ewes compared to the controls, with no significant differences in response to phenylephrine. When the dynamic (within 40 seconds) rather than the steady-state changes were analyzed, a hyperreactivity to phenylephrine was present in the pregnant animals. These data are an illustration of the complex interactions that are present in the whole-body pressor responses to vasoactive stimuli during pregnancy. (AM J OBSTET GYNECOL 1985;151:479-83.)

Key words: Vascular reactivity, blood flow, pregnancy

The fact that pregnancy as well as the steroid hormones is associated with apparent changes in vascular response to differing vasoactive stimuli has been the subject of studies in both humans and various other animal species.[1] An understanding of how vascular reactivity is changed by these different states is important to any investigation of such cardiovascular disorders as pregnancy-induced hypertension or Raynaud's syndrome.

We recently completed a study, in both pregnant and progesterone-treated ewes, that characterized the vascular responses to two different vasoactive stimuli, angiotensin II and phenylephrine, an α-adrenergic agonist. The vascular responses to these agents were examined in an acute preparation with the use of a fixed-flow hind limb perfusion technique. We found that both pregnancy and progesterone treatment were associated with a reduction in vascular reactivity to angiotensin II in this circulation. However, there was an increase in sensitivity to the vasoconstrictive effects of phenylephrine under these same two conditions. The divergent pressor response to different vasoactive stimuli illustrates the complexity of the vascular reactivity changes that must occur either during pregnancy or with a change in hormonal milieu.[2] In order to study these responses further, we decided to repeat these experiments in a chronic model in the ewe in order to verify whether these differences in vascular reactivity existed under more normal physiologic conditions.

Material and methods

Animal model. Seven pregnant mixed-breed ewes with single fetuses and six nonpregnant ewes were used in this study. The pregnant ewes had a mean gestational age of 124 + 1 day.

Following a 24-hour fast, all animals were anesthetized with halothane anesthesia. With standard, aseptic surgical techniques, catheters were placed in the external iliac artery and vein via the saphenous branches. A third arterial catheter was inserted into the circumflex artery and advanced so that the tip just entered the external iliac artery. A Dienco electromagnetic flow transducer was placed around the external iliac artery proximal to the circumflex artery catheter. The mammary artery was ligated on that side. In addition, ovariectomy was performed in the nonpregnant ewes by a midline laparotomy, and a control laparotomy was performed in the pregnant ewes as well.

With this instrumentation, the blood flow and perfusion pressure of the hind limb could be continuously monitored. These data could then be used for the calculation of the hind limb vascular resistance (millimeters of mercury per milliliter per minute). The positioning of the circumflex artery catheter allowed for a drug infusion directly into the arterial circulation to the hind limb, which helped to minimize the systemic effects of the drug.

Study design. All animals were allowed to recover from the operation for at least 1 week prior to study.

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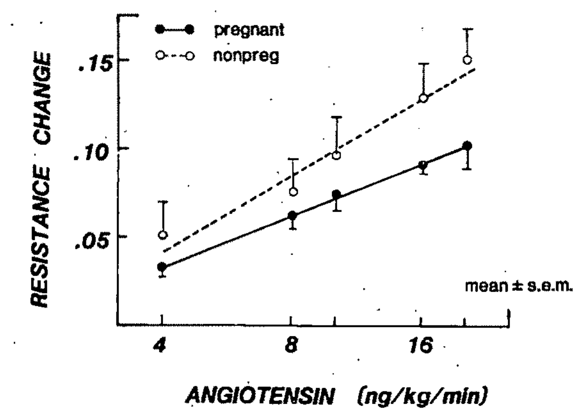

Fig. 1. Semilogarithmic plot of the change in calculated hind limb vascular resistance in response to five doses of angiotensin II in seven pregnant and six nonpregnant ewes.

During this week they were trained to the monitoring stall.

Only one drug, either angiotensin II or phenylephrine (Sigma Chemical Co.), was studied on any one study day. Following a 30-minute control period, dose-response curves were generated to either angiotensin II (4 to 20 ng/kg/min) or phenylephrine (100 to 1000 ng/kg/min). Drugs were infused at a rate of 0.1 to 0.5 ml/min directly into the circumflex artery catheter. These doses were chosen in order to produce a range of vasoconstriction in the limb with a minimal increase in systemic blood pressure. Each dose of drug was infused randomly for 5 minutes with at least 20 minutes between the angiotensin II infusions and 10 minutes between the phenylephrine infusions. All drugs were diluted in normal saline solution and kept on ice until needed.

Blood samples were drawn on the day of the study for measurement of serum sodium and potassium. These values were correlated with each animal's dose-response curve to each drug.

Data analysis. The change in hind limb perfusion pressure and external iliac blood flow with each drug dose was determined by subtracting the mean steady-state response at 3 to 5 minutes of drug infusion from the mean perfusion pressure and flow measured during the 3 minutes just prior to the drug infusion. The perfusion pressure was divided by the flow value in order to calculate the change in hind limb vascular resistance. It should be noted that a steady-state response was not always achieved at the higher doses of phenylephrine. This will be discussed in the results section. The dose-response curves for phenylephrine or angiotensin II were compared within and between groups by means of two-way analysis of variance with repeated measures and orthogonal contrasts.[5] Linear regression analysis was used to correlate serum potassium and sodium and the dose-response curves. Baseline parameters were compared by means of an unpaired Student's t test.[4]

Biophysical measurements. External iliac blood flow was measured with a Dienco flow transducer and flow meter. Arterial and venous blood pressures were monitored with Ailtech (Eaton Corp.) pressure transducers; heart rate was derived from the pulse pressure waveform on a Gould Biotach instrument. These measurements were recorded on a rectilinear recorder (Gould Instruments, Cleveland, Ohio).

Each flow transducer was calibrated after every use on the carotid artery in vivo and blood pressure transducers were calibrated periodically with a mercury manometer.

Serum sodium and potassium concentrations were determined on duplicate samples with a digital flame photometer (Model IL 443, Instrumentation Labs).

Results

Baseline parameters. Listed in Table I are the mean body weight, external iliac blood flow, baseline perfusion pressure, and calculated hind limb vascular resistance for the two groups of ewes. There was no significant difference in body weight between the two groups. There was a trend toward a lower perfusion pressure in the pregnant animals but this difference was not statistically significant. The external iliac blood flow was significantly higher in the pregnant ewes with the correspondingly lower calculated hind limb vascular resistance.

Dose response to angiotensin II. The dose responses of the change in hind limb vascular resistance caused by the administration of angiotensin II are shown in Fig. 1. The dose-response curves were linear ($p < 0.01$) over the range of doses studied and were significantly different from one another. When the results in the pregnant ewes were compared to those in the nonpregnant animals, the right-hand shift in the dose-response curve of the pregnant ewes indicates that there was a decreased sensitivity to angiotensin II in the hind limb circulation over this dose range. There was no significant correlation between the animal's dose-response curve to angiotensin II and the serum sodium and potassium levels.

Dose response to phenylephrine. Illustrated in Fig. 2 are the dose responses of the change in hind limb vascular resistance with the administration of phenylephrine in the two groups of ewes. It was found that the data were abnormally distributed in both groups. Therefore, the data were transformed by taking the negative log of the resistance change in order to perform analysis of variance on the results.[4] There was no significant difference in the change of hind limb vascular resistance to phenylephrine in these two groups of animals.

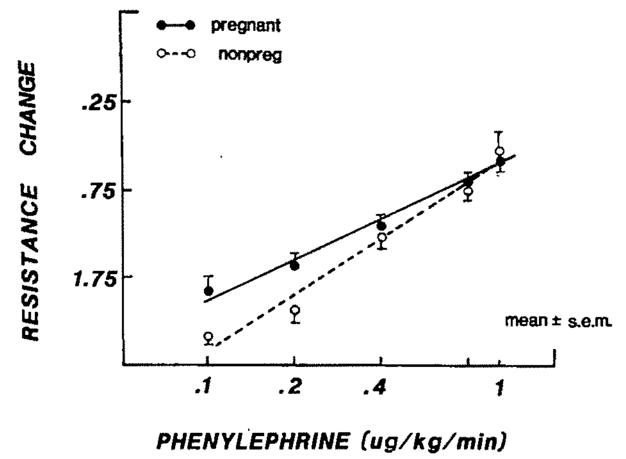

Fig. 2. A plot of the negative log of the change in calculated hind limb vascular resistance versus the log of five doses of phenylephrine in seven pregnant and six nonpregnant ewes.

Table I. Baseline parameters

	Nonpregnant ewes	*Pregnant ewes*
Weight (kg)	47 ± 2	48 ± 1
Gestation (days)		124 ± 1
Perfusion pressure (mm Hg)	76 ± 5	71 ± 4
Flow (ml/min)	241 ± 25	337 ± 43*
Resistance (mm Hg/ml/min)	0.334 ± 0.05	0.229 ± 0.03*

Values are mean ± SEM.
*$p < 0.05$.

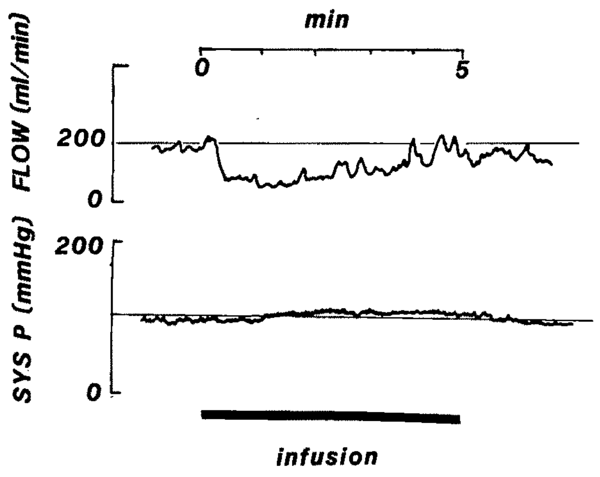

Fig. 3. A tracing of a record from a pregnant ewe showing the hind limb blood flow and systolic pressure during an infusion of phenylephrine (1 ng/kg/min).

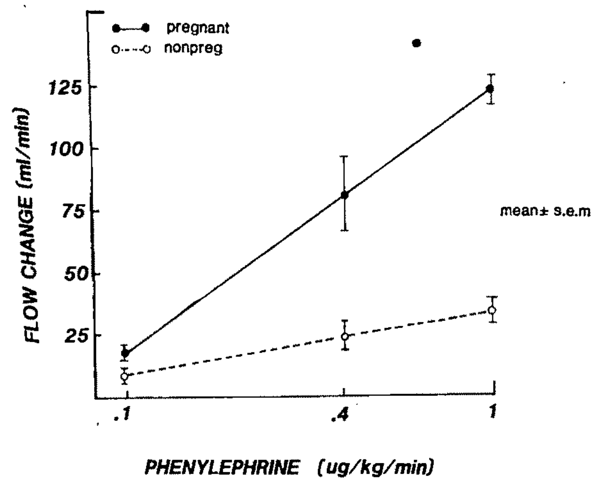

Fig. 4. Semilogarithmic plot of the change in hind limb blood flow during the first 40 seconds of infusion of three doses of phenylephrine in seven pregnant and six nonpregnant ewes.

Since these observations were dissimilar to the observations from our earlier acute study in which the flow to the hind limb was fixed, we reexamined the dynamic rather than the steady-state response to the drug infusions. Illustrated in Fig. 3 is a tracing of one record from a pregnant ewe showing the blood flow and systolic blood pressure response to the highest dose of phenylephrine. The decrease in blood flow preceded the increase in systolic pressure by about 20 to 40 seconds. As the pressure began to rise there was a tendency for the flow to recover. Indeed, at this dose a steady-state response had not been reached by the end of a 5-minute infusion. This dynamic change in external iliac blood flow in response to the highest dose of phenylephrine in the pregnant ewes was found to be much more marked than that of the control ewes during the initial portion of the infusion period. Subsequently, the data were reanalyzed with the change in external iliac blood flow prior to a change in pressure used as the dependent variable. The mean dose-response curves for the two groups of ewes are depicted in Fig. 4. The pregnant ewes had a significantly greater reduction ($p < 0.03$) in blood flow in response to phenylephrine administration than the nonpregnant ewes. This same relationship occurred when the data were calculated as the change in hind limb vascular resistance since the flow response was occurring prior to a significant alteration in the systemic blood pressure response.

As with angiotensin II, there was no correlation between the animal's dose-response curve to phenylephrine and the serum sodium and potassium levels.

Comment

The data in this study are, in essence, consistent with our previous observations that were obtained with an acute, fixed-flow hind limb perfusion model.[2] Pregnancy is associated with a reduction in sensitivity to the vasoconstrictor effects of angiotensin II in this circulation in the ewe. In addition, the vascular response to the vasoconstrictive effects of phenylephrine, in α-adrenergic agonist, is increased in the same model. The mechanisms that may be involved in these associations have been discussed in detail elsewhere.[1, 2, 5-8] An additional parameter that was examined in this study was the serum sodium and potassium levels. Sodium has been found to modulate angiotensin II receptor affinity, and minor alterations in plasma potassium can alter the resting membrane potential of smooth muscle.[9] There was no apparent correlation between these baseline levels and an individual animal's dose response curve. It may be necessary to manipulate these levels artificially in order to demonstrate a clear-cut relationship between them and a dose-response curve from the type of model used in this study.

The difficulties that were involved in assessing vascular reactivity in a chronic model in this study warrant some discussion. The most appropriate way to evaluate the vascular sensitivity to a stimulus is to use one index of vessel tone, either flow or pressure, as the dependent variable while the other parameter is held constant. In addition, the pressure-flow characteristics of the circulation should be established and taken into consideration in the final data analysis.[10] Obtaining complete pressure-flow curves is time consuming and can only be performed in an acute preparation. As a compromise, drug dose-response curves are obtained in the acute model by either a fixed-flow or a fixed-pressure technique[11] Obviously, in the chronic model it is not possible to fix either the flow or the pressure. One must rely on the calculation of the vascular resistance of the circulation with a change in this value in response to a stimulus used as an index of vascular reactivity. Again, the optimal situation is one in which either the flow or the pressure remains relatively constant during the test period. This helps to minimize the variability in response that may exist between animals or groups because of potential differences in the pressure-flow characteristics of the circulation. Pregnancy is known to affect this relationship in the rabbit,[12] but this parameter has not been studied in the sheep. Keeping systemic pressure constant will avoid pressure-flow interactions and, in addition, minimize baroreflex effects upon the skeletal bed as the sensitivity of this system could change during pregnancy.

In the study reported here we attempted to keep the drug dose to the hind limb low enough that there was little change in the systemic blood pressure but a demonstrable change in the hind limb blood flow. The doses chosen to meet these criteria in the angiotensin II study were such that the hind limb vascular resistance changes were relatively small in comparison to those in the phenylephrine study. The problem with the phenylephrine study was that the decreases in flow in response to the drug were as high as 70% of the resting flow. By 3 to 5 minutes of drug infusion the flow began to return toward the control level, probably because of the accumulation of local metabolites, which resulted in vasodilation of sufficient magnitude to overcome partially the direct drug effect. In addition, at the higher drug doses, there was some change in systemic blood pressure, with the pressure in the nonpregnant ewes increasing more than that in the pregnant ewes. Therefore, the calculated hind limb vascular resistance change at 3 to 5 minutes of infusion was the sum of all these interactions rather than the more direct vascular response to the drug itself. We recalculated the changes during the first 20 seconds of drug infusion in order to minimize these influences. There was a striking difference in the immediate flow response to phenylephrine administration between the pregnant and nonpregnant ewes during a period of no pressure change. This emphasizes the complex interactions occurring at the 3- to 5-minute portion of the drug infusion. By this time, the change in calculated hind limb vascular resistance was equivalent between the two groups, a situation that did not exist at the initiation of the infusion.

In addition to the problem of pressure-flow interactions there is a difficulty in deciding how to calculate the dose to administer each animal. We chose to calculate the dose on a body weight basis because in our previous acute study this method provided data with the least within-group variability. Since the flow was fixed in the acute study, the concentration of drug per milliliter of blood flow could be calculated. With that approach, the same drug-sensitivity relationships were observed between groups although the within-group variability increased. However, in pregnancy there is an increase in body weight that may or may not be reflected by a proportional increase in the weight of the hind limb. Therefore, it is possible that the hind limbs of the pregnant ewes received more total drug on a tissue weight basis even though there was no significant difference in body weight between the two groups. On the other hand, the higher hind limb blood flow in the pregnant animals may have had a dilutional effect. The fact that the vascular resistance changes were decreased in the pregnant animals with one vasoconstrictor but increased with another, when compared to results in the controls, suggests that in spite of these variables the direction of the change is a real difference. The best means of data analysis would be to remove the limb at the end of the study, then calculate the resistance changes based upon milliliters of blood flow per 100 gm of tissue. One could then calculate the amount of drug actually received on a hind limb weight basis. This would significantly reduce the between-animal variability that we have observed in these particular vascular responses. This approach is presently being pursued in our laboratory.

In summary, these data are consistent with our previous findings from an acute preparation that demonstrate that pregnancy is associated with a divergent pressor response in the hind limb of the ewe to two different vasoactive stimuli. However, use of the chronic model increases variability in the data because of the difficulty in controlling various hemodynamic parameters such as the perfusion pressure at the time of infusion. The difficulties which do exist in studying vascular reactivity during pregnancy in a chronic model do not negate the importance of examining these issues in a more physiologic condition.

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Hemodynamic effects of indomethacin in chronically instrumented pregnant sheep

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Indomethacin administration has produced decreases in uteroplacental blood flow in several animal studies; therefore, it has been suggested that the maintenance of uterine blood flow is critically dependent on the continued synthesis of vasodilating prostaglandins. However, vasoconstriction following indomethacin administration may be due to mechanisms other than reduced prostaglandin synthesis. We administered indomethacin (2, 5, or 10 mg/kg) intravenously to seven unanesthetized sheep in late pregnancy and determined the time courses of the uteroplacental and systemic hemodynamic responses, comparing these to the concurrent changes in circulating prostaglandins. Indomethacin administration resulted in rapid increases in systemic and uteroplacental vascular resistance (80% to 100%) and mean arterial pressure (~30%) and in decreases in systemic (~30%) and uteroplacental (0% to 30%) blood flows within 5 minutes. Vasoconstriction was transient, however, and after 60 minutes there was no evidence of uterine or systemic vasoconstriction, although systemic and uterine plasma prostaglandin levels remained reduced for 180 minutes. Thus substantial inhibition of prostaglandin synthesis existed without evidence of concurrent systemic or uteroplacental vasoconstriction, suggesting that uterine blood flow is not directly dependent on maintained prostaglandin synthesis in unstressed pregnant sheep. Furthermore, the transient indomethacin-induced vasoconstriction may not be due to inhibition of prostaglandin synthesis. (Am J Obstet Gynecol 1985;151:484-93.)

Key words: Uterine blood flow, cardiac output, prostaglandins

Prostaglandins have been reported to be produced in substantial amounts in the uterus and placenta of pregnant animals,[1-3] and various prostaglandins have been shown to induce uterine vasodilation when infused directly into the uterine vasculature.[4-7] These observations have led to the speculation that vasodilating prostaglandins may have an important role in maintaining the substantial increase in uterine blood flow which occurs in pregnancy.

If uterine blood flow is dependent on the continued production of vasodilating prostaglandins, it might be expected that the reduction of endogenous prostaglandin levels would result in a parallel fall in uteroplacental perfusion. Several investigators have attempted to evaluate this by administering indomethacin to inhibit prostaglandin synthesis in vivo, but variable results have been reported. In some studies, indomethacin treatment did result in significant decreases in uteroplacental blood flow.[2, 3, 8, 9] However, Gerber et al.[10] found that indomethacin led to a 35% increase in uterine blood flow, and Speroff et al.[1] observed both increases and decreases in uterine blood flow in the same series of monkeys. Furthermore, McLaughlin et al.[11] observed uterine vasoconstriction following indomethacin which was remarkably transient, lasting less than 25 minutes. Although prostaglandin levels were not measured in their study, this transient vasoconstriction is surprising, as it would be expected from other studies[12] that prostaglandin synthesis would have been suppressed for 4 to 6 hours or longer and that uterine blood flow would have remained low for a similar period.

It is possible that this transient uterine vasoconstriction reflected an unusually brief effect of indomethacin on prostaglandin synthesis in the pregnant sheep. However, if reduced prostaglandin synthesis could be shown to exist without parallel uteroplacental vasoconstriction, then the hypothesis that uteroplacental blood flow is critically dependent on the level of prostaglandin synthesis would appear less tenable. Furthermore, it is not clear whether the increases in uterine blood flow observed in some studies[1, 10] may have been due to concurrent systemic vascular responses to indomethacin causing, for example, an increase in arterial pressure. In order to clarify these issues, we have administered

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indomethacin to a group of chronically instrumented pregnant sheep and have evaluated the uterine hemodynamic responses in relation to the concurrent changes in uterine and systemic prostaglandin levels and to the overall systemic vascular responses. Because of the possible involvement of the renin-angiotensin system in the hemodynamic responses, plasma renin activity was also measured.

Material and methods

The complicating effects of anesthesia and recent operation on vascular responses are well established[13-15]; therefore, only chronically instrumented animals remote in time from operation were used. Seven ewes of mixed breed were studied in late pregnancy, between 130 and 141 days' gestation (term, 144 ± 3 days). The animals' weights ranged from 37 to 59 kg at the time of study (51 ± 7.3 kg, mean ± SD). All fetuses (four singletons and three sets of twins) were delivered alive and healthy or were alive several days after the indomethacin experiments, at which time the ewe was put to death for other (microsphere) studies. The animal preparation has been described in detail previously.[16] Briefly, at operation (~125 days) electromagnetic flow probes (Micron Instruments, Inc., Los Angeles, California) were implanted around both main uterine arteries, and polyvinyl catheters were inserted into both femoral arteries to the level of the trifurcation of the abdominal aorta and into both femoral veins, with the open ends lying in the inferior vena cava just below the diaphragm. In some animals, catheters were also introduced into the uterine vein of a pregnant horn via a peripheral tributary. The flow probes and catheters were brought out to the flank through a subcutaneous tunnel and maintained in a canvas pouch attached to the skin with steel pins. Catheters were flushed daily with heparinized saline (250 U/ml) and closed with sterile pins. Penicillin (600,000 U) and streptomycin (0.5 gm) were given on the day of operation and the two subsequent days.

The ewes were maintained after operation in individual stalls kept in the laboratory. They were given standard animal chow (Purina Commercial Creep Chow II, G) and water freely throughout the study period. Body weight remained constant or increased. The animals were studied only after they were considered to have recovered from surgical trauma and anesthesia, at or beyond the seventh day after operation. Each animal was studied with one to three doses of indomethacin; consecutive studies were performed not less than 3 days apart.

Experimental protocol. Three doses of indomethacin (lyophilized sodium indomethacinate, Merck Sharp & Dohme, West Point, Pennsylvania) were used: 2 mg ($n = 6$), 5 mg ($n = 4$), or 10 mg ($n = 4$) per kilogram of body weight, where n is the number of experiments. The total dose was dissolved in 20 ml of sterile isotonic saline. After a 30-minute control period, the indomethacin was infused into the vena cava by a Harvard infusion pump at a constant rate during 5 minutes and the catheter flushed at the same rate. Serial blood samples and hemodynamic measurements were obtained before, during, and for 4 hours after the indomethacin infusion.

Hemodynamic measurements. Mean arterial pressure in the lower abdominal aorta was monitored continuously from one arterial catheter with a pressure transducer (Type 4-327-1019, Bell and Howell, Pasadena, California) and the signal recorded on a two-channel pen recorder (Model 220, Brush Instruments Div., Clevite Corp., Cleveland, Ohio.). Uterine blood flow was monitored with square-wave electromagnetic flowmeters (Model RC 1000, Micron Instruments, Inc., Los Angeles, California) and recorded on a second, integrated, two-channel recorder; the sum of the simultaneous measured flows in both main uterine arteries was used for analysis. Cardiac output was measured by the dye-dilution technique. Five milligrams of indocyanine green (Cardio-Green, Hynson, Westcott & Dunning, Inc., Baltimore, Maryland) was injected rapidly into the inferior vena cava via one indwelling catheter while blood was withdrawn at 10 ml/min through a densitometer from a femoral arterial catheter. This blood was later reinfused into the animal. Optical density was recorded graphically and cardiac output calculated by electronic integration directly from the signal. The results of cardiac output calculated electronically by this equipment (Cardiac Output Recorder, COR-100A, Waters Instruments, Inc., Rochester, Minnesota) have been used in this report; the validity of these measurements has been reported.[17] All hemodynamic variables were stable during the 30-minute control period; measurements of each variable were obtained at 5-minute intervals and an average control value determined. After the indomethacin infusion was begun, observations were recorded at 1, 3, 10, 20, 40, and 60 minutes and at 30-minute intervals thereafter.

Prostaglandin measurements. Blood samples for prostaglandin assay were taken during the control period and at 5, 20, and 180 minutes after the start of the indomethacin infusion. Six milliliters of blood was rapidly withdrawn from a vena caval catheter into a chilled plastic syringe containing 60 μg of indomethacin to inhibit further prostaglandin synthesis. In five experiments blood samples were also taken simultaneously from catheters in the uterine vein. The blood was immediately transferred to iced tubes containing 100 U of heparin and centrifuged at 1° C for 10 minutes at 750 × g. Aliquots of 3 ml of plasma were then stored at −18° C until assayed for prostaglandins. Prosta-

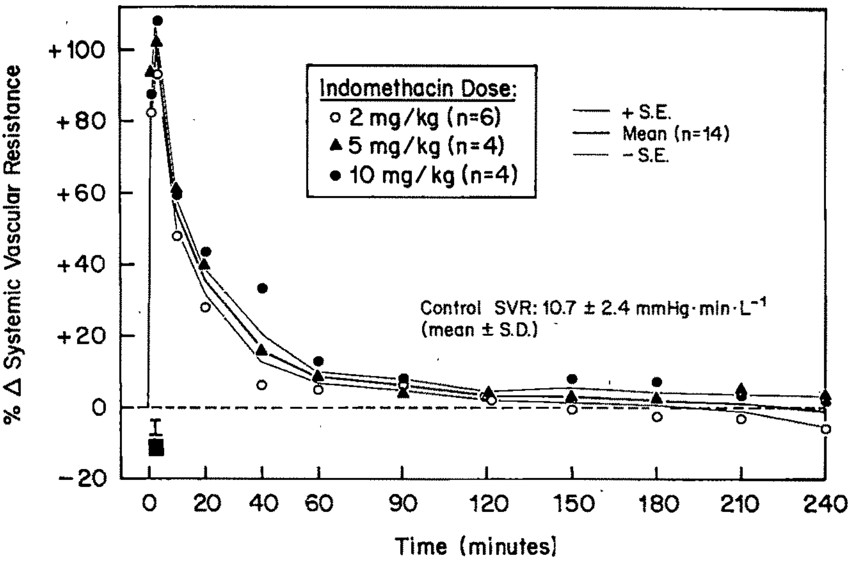

Fig. 1. The response of systemic vascular resistance *(SVR)* to systemically infused indomethacin *(I)*. Responses are expressed as percentages of control values; the *points* (○, △, ●) represent the mean of responses for the three doses of indomethacin and the lines represent the mean ± SE for all 14 experiments. The mean response (n = 14) is statistically significant ($p < 0.05$) from 1 through 40 minutes.

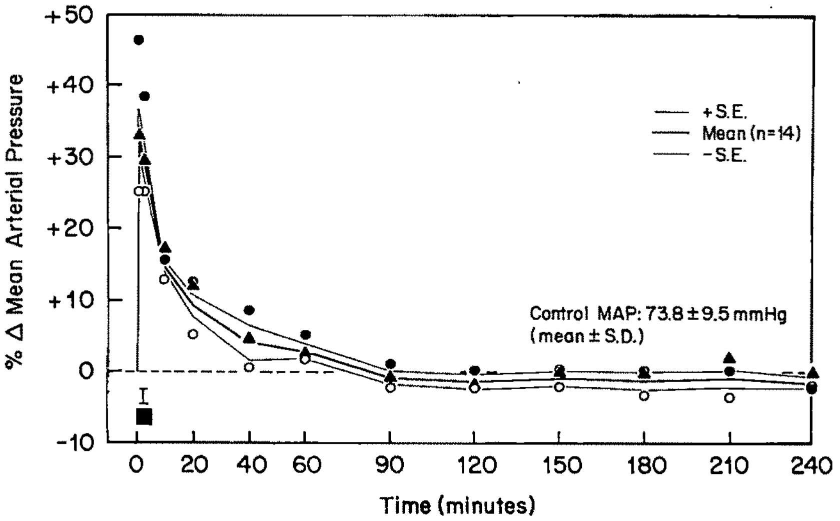

Fig. 2. The response of mean arterial pressure *(MAP)* to systemically infused indomethacin *(I)*. Responses are expressed as percentages of control values; the *symbols* are as described in the legend to Fig. 1. The mean response (n = 14) is statistically significant ($p < 0.05$) from 1 through 20 minutes.

glandins E_2 and $F_{2\alpha}$ in plasma were measured by the radioimmunoassay method of Dray et al.[18] as modified by Campbell et al.[19] The sensitivity of the assay was <5 pg per tube, recoveries averaged 85%, and the inter-assay and intra-assay variabilities were 9% and 6%, respectively.

Plasma renin activity. Serial arterial blood samples were obtained and processed as above except that indomethacin was not added to the syringe. Plasma renin activity was measured as angiotensin I generation (RIANEN, New England Nuclear) and expressed as nanograms of angiotensin I formed per milliliter of plasma per hour. The use and accuracy of this assay in our laboratories have been reported.[20]

Data analysis. As increases in uterine blood flow may be produced by either uterine vasodilation or an increase in uterine perfusion pressure, we have calculated uterine vascular resistance in these studies. This allows for the effect of increased perfusion pressure in examining the response of the uterine vasculature.

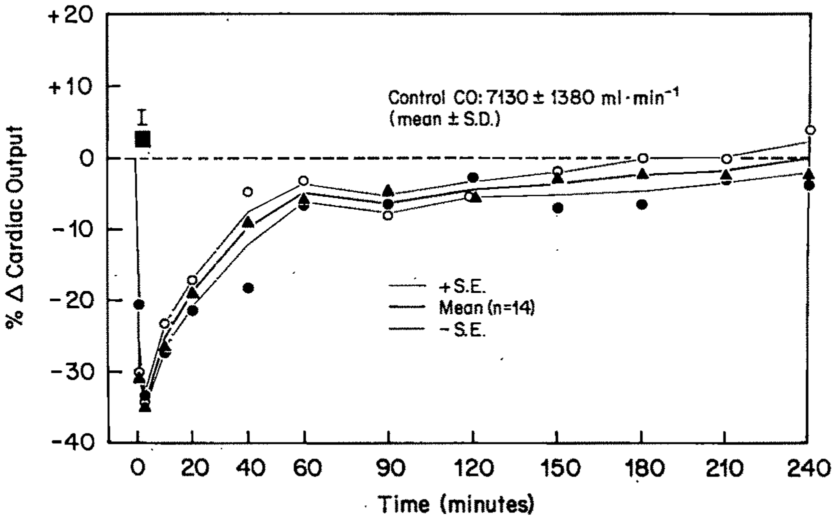

Fig. 3. The response of cardiac output *(CO)* to systemically infused indomethacin *(I)*. Responses are expressed as percentages of control values; the *symbols* are as described in the legend to Fig. 1. The mean response (n = 14) is statistically significant ($p < 0.05$) from 1 through 40 minutes.

Uterine vascular resistance may be calculated as the difference between mean arterial and venous pressure, divided by uterine blood flow. Intrauterine and systemic venous pressures have been measured and both found to be consistently low, 3 to 5 and 1 to 3 mm Hg, respectively. As these pressures are very small compared to mean arterial pressure, they were not included in the calculation of uterine vascular resistance. Thus uterine vascular resistance was calculated as $UVR_t = MAP_t/UBF_t$, where UVR = uterine vascular resistance, MAP = mean arterial pressure, UBF = uterine blood flow, and t is the same point in time.

Total systemic vascular resistance was calculated as the mean arterial pressure divided by cardiac output, which represents simultaneous total systemic blood flow. As above, since venous pressure is small, systemic vascular resistance was calculated as $SVR_t = MAP_t/CO_t$, where SVR = systemic vascular resistance, MAP = mean arterial pressure, CO = cardiac output, and t is the same point in time.

It was noted that baseline values of the hemodynamic variables varied considerably between animals and, although the responses observed following indomethacin were consistent, the absolute changes varied in magnitude, depending on the baseline values. Thus the changes following indomethacin have been standardized by expressing all changes as a percentage of control values. This transformation also allows comparison of the relative magnitude of the responses in the different hemodynamic variables.

All data were analyzed by repeated-measures analysis of variance with respect to time and to the dose of indomethacin. Where statistical significance was observed ($p < 0.05$), this was further analyzed by Duncan's Multiple Range Test.[21] For the reasons noted above, only the data expressed as percentage changes are presented; however, statistical analyses were also performed on the raw data and the results were entirely consistent with those for the data presented.

Results

Hemodynamic responses. The hemodynamic responses during and following the infusion of indomethacin are shown in Figs. 1 to 6; the points representing the mean data for each dose are illustrated. Except for a lesser response in uterine blood flow and uterine vascular resistance at 3 and 10 minutes with an indomethacin dose of 2 mg/kg, there were no significant differences in the hemodynamic responses to the different doses of indomethacin; therefore, the data have also been combined and presented as the mean (±SE) for all 14 experiments. Absolute responses can be estimated from the control data presented in each figure.

Within the first minute of initiation of the indomethacin infusion there was a rapid rise in systemic vascular resistance (Fig. 1), reaching a maximum increase of 100% ± 6% within the first 3 minutes. This rise in systemic vascular resistance was reflected in an increase in mean arterial pressure of 34% ± 2% (Fig. 2) and was associated with a simultaneous fall in cardiac output of 34% ± 2% (Fig. 3). Hemodynamic variables after 40 minutes were not statistically different from control values, and by 120 minutes systemic vascular resistance, mean arterial pressure, and cardiac output had returned to within 5% of control levels. The cardiac responses to indomethacin are further illustrated in Fig. 4, where heart rate is seen to fall in parallel with

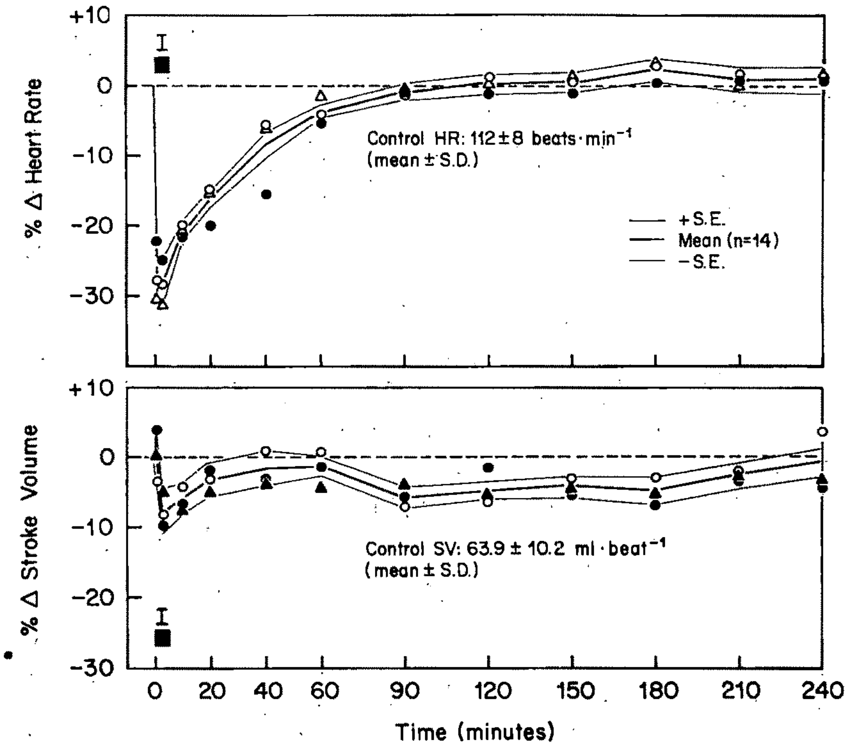

Fig. 4. The response of heart rate *(HR)* and stroke volume *(SV)* to systemically infused indomethacin *(I)*. Responses are expressed as percentages of control values; the *symbols* are as described in the legend to Fig. 1. The mean response ($n = 14$) in *HR* is statistically significant ($p < 0.05$) from 1 through 40 minutes; the mean response in *SV* is not statistically significant.

cardiac output. Although stroke volume decreased, this change was minimal and was not statistically significant.

The response of uterine vascular resistance (Fig. 5) was similar to that in systemic vascular resistance, a rapid rise reaching a peak of 52% ± 11% at about 5 minutes. However, the increase in vascular resistance in the uterus was even more short-lived than that seen in the systemic vasculature, uterine vascular resistance having returned to control levels by 20 minutes, whereas systemic vascular resistance remained elevated for at least 40 minutes. Changes in uterine blood flow also were very transient (Fig. 6) in contrast to changes in total systemic blood flow (Fig. 3); after 20 minutes uterine blood flow was slightly increased from control levels ($p > 0.05$). The apparently greater increase in uterine blood flow seen during the steady state with 10 mg/kg of indomethacin was not statistically significant.

Plasma prostaglandins. The concentrations of prostaglandins E_2 and $F_{2\alpha}$ in vena caval plasma are presented in Table I for experiments in which values at all four sampling times were available. By 20 minutes the levels of both prostaglandins E_2 and $F_{2\alpha}$ had fallen significantly from control levels and this fall, approximately 50%, persisted without significant change through 180 minutes. There was no difference in the changes in prostaglandin concentrations with the different doses of indomethacin.

In five experiments, plasma prostaglandins were measured in simultaneous samples of blood obtained from the vena cava and uterine vein; these results are presented in Table II. In the control period, the concentration of prostaglandin E_2 in the uterine vein was significantly higher than in the vena cava ($p < 0.05$), but the difference in prostaglandin $F_{2\alpha}$ levels was not statistically significant. Except for a greater decrease in vena caval prostaglandin $F_{2\alpha}$ at 5 minutes, the changes in plasma prostaglandins observed after indomethacin were similar in the uterine vein and vena cava. Again there was no apparent difference in these changes in prostaglandin concentration with the different doses of indomethacin.

Early response to indomethacin. The change in uterine blood flow within the first 5 minutes after the infusion of indomethacin was begun followed a distinctive pattern which is illustrated in Fig. 7, *A*. The changes in uterine blood flow were determined by the relative changes in uterine perfusion pressure (effectively mean arterial pressure) and in uterine vascular resistance. Although the early uterine vascular responses to indomethacin appeared to be dose-related, the responses in all experiments were qualitatively similar. Thus, in order to illustrate the pattern of these responses more clearly, we have chosen to present the data obtained only with the highest dose of indometh-

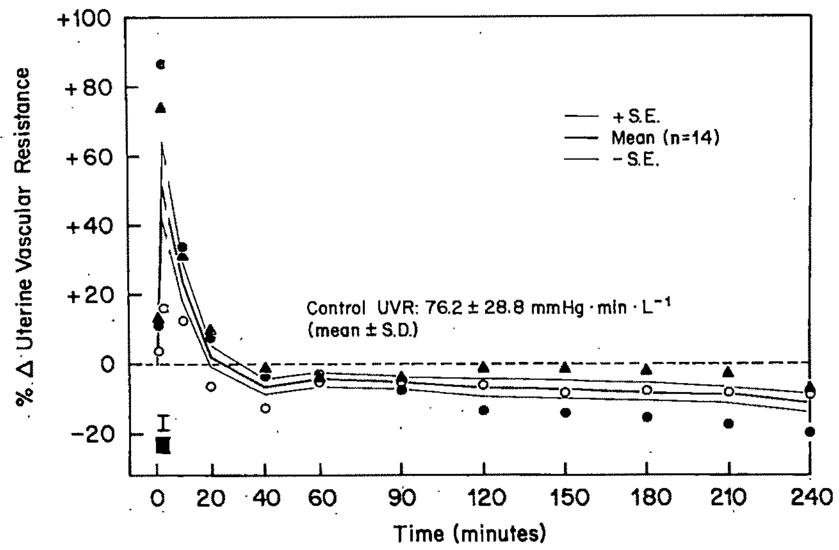

Fig. 5. The response of uterine vascular resistance *(UVR)* to systemically infused indomethacin *(I)*. Responses are expressed as percentages of control values; the *symbols* are as described in the legend to Fig. 1. The mean response (n = 14) is statistically significant ($p < 0.05$) from 3 through 10 minutes.

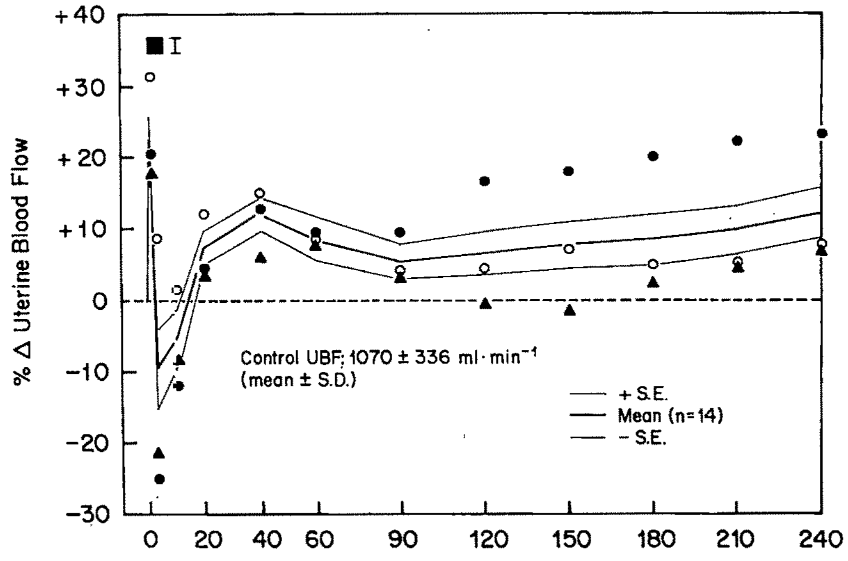

Fig. 6. The response of uterine blood flow *(UBF)* to systemically infused indomethacin *(I)*. Responses are expressed as percentages of control values; *symbols* are as described in the legend to Fig. 1. The mean response (n = 14) was statistically significant ($p < 0.05$) at 1, 3, 4, and 5 minutes only.

acin, 10 mg/kg (Fig. 7, *A*). It can be seen that mean arterial pressure rose rapidly, reaching a maximum within 1 minute of the beginning of indomethacin; in contrast, the increase in uterine vascular resistance was delayed, not reaching a maximum until 4 to 5 minutes. The delay in the response of uterine vascular resistance was reflected by a rise in uterine blood flow, which peaked at ~1 minute, when the substantial increase in mean arterial pressure (46%) was offset by only a small rise in uterine vascular resistance (11%). Subsequently, however, mean arterial pressure changed little while uterine vascular resistance continued to rise and thus uterine blood flow fell, reaching a nadir at ~5 minutes when the rise in mean arterial pressure (32%) was considerably outweighed by the greater increase in uterine vascular resistance (115%). In Fig. 7, *B*, we have presented for comparison the responses of these same three variables during the first 5 minutes of a constant infusion of angiotensin II (11.5 μg/min) into the vena cava of seven ewes, studied similarly in late pregnancy. The data from which this figure is derived have been presented in detail in a previous publication.[17] The same biphasic response of uterine blood flow was seen and was related to the delay in the increase in uterine

Table I. Systemic venous plasma prostaglandin levels before and after indomethacin (2 to 10 mg/kg)

Prostaglandin	*n*	*Control*	*Time after onset of indomethacin infusion*		
			5 min	*20 min*	*180 min*
E_2 (pg/ml)	14	214 ± 18	200 ± 26	135 ± 19*	112 ± 10*
%Δ			−6 ± 8	−36 ± 6	−45 ± 5
$F_{2\alpha}$ (pg/ml)	10	180 ± 17	111 ± 9*	87 ± 19*	65 ± 10*
%Δ			−37 ± 3	−50 ± 9	−57 ± 12

Values are mean ± SE. %Δ = % Change from control.

*Values for each prostaglandin which are different from control ($p < 0.05$) but not statistically different from each other.

Table II. Plasma prostaglandin levels in the vena cava and uterine vein before and after indomethacin (2 to 10 mg/kg)

Prostaglandin	*Control*	*Time after onset of indomethacin infusion*		
		5 min	*20 min*	*180 min*
E_2 (pg/ml)				
Vena cava	233 ± 30	222 ± 48	181 ± 47	113 ± 18*
%Δ		−5 ± 14	−27 ± 11	−42 ± 11
Uterine vein	458 ± 67	531 ± 109	225 ± 28*	197 ± 22*
%Δ		+13 ± 6	−50 ± 5	−54 ± 7
$F_{2\alpha}$ (pg/ml)				
Vena cava	188 ± 4	112 ± 5*	126 ± 29*	66 ± 14*
%Δ		−40 ± 3	−41 ± 11	−64 ± 7
Uterine vein	223 ± 22	191 ± 22	90 ± 23*	105 ± 11*
%Δ		−13 ± 8	−58 ± 12	−52 ± 5*

Values are mean ± SE (n = 5). %Δ = % Change from control.

*Values for each prostaglandin which are different from control ($p < 0.05$) but not statistically different from each other.

vascular resistance compared to the increase in mean arterial pressure.

Plasma renin activity. In view of the possible involvement of the renin-angiotensin system in the hemodynamic responses observed following indomethacin, we also measured arterial plasma renin activity before and serially after the administration of indomethacin. There were no differences in the changes in plasma renin activity with the three doses of indomethacin studied; thus the data for all 14 experiments have been combined. The plasma renin activity fell significantly from a baseline level of 2.38 ± 0.15 to 1.78 ± 0.14 ng/ml · hr by 5 minutes after the infusion of indomethacin was begun. By 20 minutes plasma renin activity had decreased further to 1.42 ± 0.14 ng/ml · hr and was unchanged through 60 minutes. By 120 minutes the levels of plasma renin activity had increased to 1.96 ± 0.19 ng/ml · hr, a significant increase from levels observed at 20 and 40 minutes ($p < 0.05$) but still significantly below control values. Plasma renin activity remained at this level through 240 minutes.

Comment

The present study was designed to investigate the effects of indomethacin on both the systemic and the uteroplacental hemodynamics of the sheep in late pregnancy. By measuring plasma prostaglandin levels serially throughout the experiments, we have attempted to relate the time course of the hemodynamic responses to that of prostaglandin synthesis inhibition. From this paradigm we hoped to gain further insight into the role of changes in prostaglandin synthesis in mediating the hemodynamic responses to indomethacin and the role of prostaglandins in the maintenance of uteroplacental perfusion in normal pregnancy.

We observed in these studies that indomethacin administration to the chronically instrumented ewe in late pregnancy produced marked systemic and uteroplacental vasoconstriction. However, it is notable that this vasoconstriction was only short-lived, as previously observed by McLaughlin et al.,[11] and that the effect on the uteroplacental vasculature was even briefer than that seen in the overall systemic vasculature. In both studies, there was no evidence of significant vasoconstriction after 40 minutes from the administration of indomethacin, a finding incongruent with the duration of prostaglandin synthetase inhibition expected from previous in vitro and in vivo observations.[12]

The effects of prostaglandins on organ perfusion are probably determined mainly by their in situ synthesis in vascular tissue rather than by their levels in circulating blood, with the possible exceptions of prostacyclin or prostaglandin D_2. However, if changes in plasma prostaglandin concentrations are in any way reflective of changes in prostaglandin synthesis in vascular tissues, the present data are evidence that indomethacin produces a substantial reduction in endogenous prostaglandin production. It is of note that this suppression of prostaglandins persisted for at least 180 minutes after indomethacin administration, that is, well beyond the time when all hemodynamic evidence of vasoconstriction had disappeared. Therefore, it appears that substantial suppression of prostaglandin synthesis may be present without any associated reduction in either uteroplacental or overall systemic blood flows. This is supportive of the view that, in the unstressed pregnant sheep, uteroplacental blood flow is not directly related to the level of overall prostaglandin synthesis.

In our study, inhibition of prostaglandin synthesis appears to have been incomplete, and it is possible that the indomethacin did not suppress endogenous tissue prostaglandins to a degree sufficient to interfere with their postulated vasodilator role. While this remains a theoretical possibility, a range of doses of indomethacin was used and the highest dose, 10 mg/kg, was as high as in previous studies, except for that of Terragno et al.[2] who infused up to 120 mg/kg, doses that may well have had toxic effects. Furthermore, we found that the reduction of circulating prostaglandins was not dose-related above 2 mg/kg. This incomplete suppression of prostaglandin synthesis by indomethacin contrasts markedly with the almost total inhibition of prostaglandin synthetase seen in in vitro studies. However in most in vivo studies, as in the present report, the decrease in plasma prostaglandin concentrations was rarely more than 60%.[12] From these observations it appears that in vivo the maximum extent of prostaglandin synthesis inhibition by indomethacin is found at a dose of 2 mg/kg, as reported by others,[22] and that drug dosage is not a significant factor in the difference between our findings and those in previous reports. Furthermore, although inhibition of prostaglandin synthesis may have been incomplete, it was nevertheless substantial, and uteroplacental perfusion appears not to be as sensitive to decreases in prostaglandin synthesis as might have been expected from earlier studies.

It is possible that the synthesis of other prostaglandins not measured in our studies, for instance, prostacyclin, may not have been significantly inhibited by indomethacin, or that their inhibition was more evanescent than for those measured, or that the inhibition of vasodilator prostaglandins occurred more rapidly than that of the vasoconstrictors. These possibilities seem unlikely, in that indomethacin is believed to act primarily by reducing the production of the endoperoxides prostaglandin G_2 and prostaglandin H_2. These compounds are presently considered to be the common substrates for all known active prostaglandins, and thus it is likely that the synthesis of all active prostaglandins would be reduced similarly.

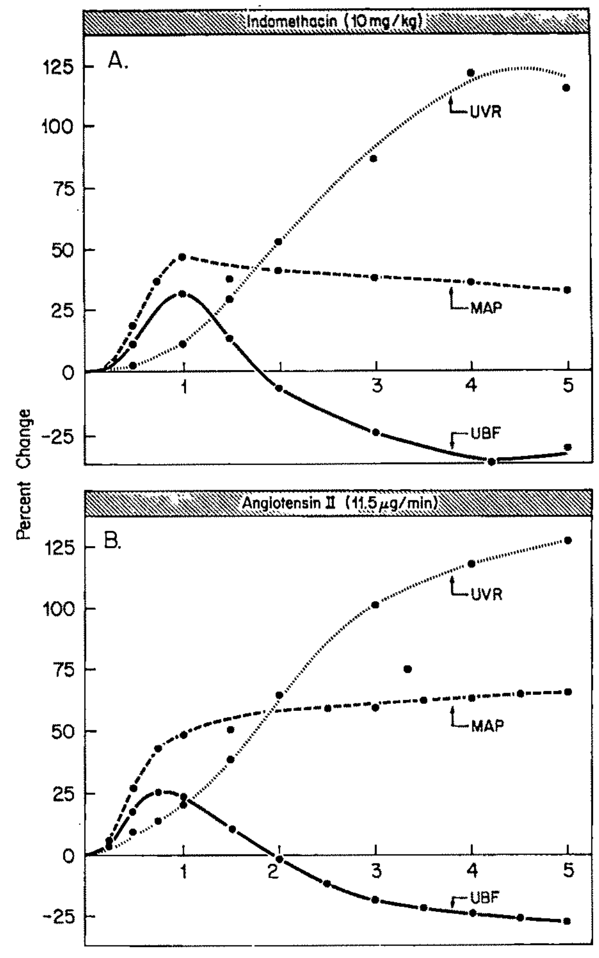

Fig. 7. Comparison of the relative responses in *UBF*, *MAP*, and *UVR*, expressed as percentages of control values, during the first 5 minutes of constant, systemic infusions of *(A)* indomethacin (10 mg/kg) and *(B)* angiotensin II (11.5 µg/min). *Points* represent the means of four and seven experiments for indomethacin and angiotensin II, respectively.

What then are possible explanations for the differences between our results and those in previous reports? The importance of prostaglandins in the maintenance of regional blood flow appears to vary with the state of the animal. This has been clearly shown in relation to the renal circulation, where inhibition of prostaglandin synthesis produces no significant change in renal blood flow in chronically instrumented animals,[23] whereas substantial falls in renal blood flow occur when the animals are concurrently exposed to laparotomy,[24] volume/salt depletion,[25] or other states in which plasma angiotensin II is increased.[26] Similar variables likely were present in previous studies in which indomethacin was observed to have induced uteroplacental vasoconstriction[1-3, 8, 9]; in contrast, we have allowed adequate time for postoperative recovery and return to normal food intake and sodium/fluid balance. Interestingly, Gerber et al.[10] reported a 35% increase in uteroplacental blood flow after indomethacin treatment in laparotomized dogs. This could reflect an interspecies difference or perhaps could be an artifact of the indirect method of measuring uteroplacental blood flow used by these investigators. However, it is also possible that the time after indomethacin administration at which uteroplacental blood flow is measured may be an important factor. We have observed (Fig. 6) that the response in uterine blood flow following indomethacin has three phases, which is in agreement with the observations of McLaughlin et al.[11] Most other authors have reported only single measurements of uterine blood flow after indomethacin; where it is possible to determine the timing of these observations, the reported changes in uterine blood flow are consistent with the responses observed in the present study. For example, Gerber et al.[10] measured uteroplacental blood flow 30 minutes after indomethacin administration, at which time we too observed a consistent increase in uteroplacental blood flow (Fig. 6). In contrast, Anderson et al.[8] and Berssenbrugge et al.[9] reported decreased uteroplacental blood flow at or before 20 minutes, by which time uteroplacental blood flow may not have recovered from the transient early fall seen in our studies (Fig. 6). Interpretation of data is difficult when observations of hemodynamic responses are made at isolated points in time without a description of the time course of the responses, especially when variables are changing; therefore, general conclusions from such data concerning a steady state must be questionable. In our study a relative steady state was observed from 60 to 240 minutes, and our conclusions so far have been largely based on observations from this period.

Although we observed no significant evidence of systemic or uteroplacental vasoconstriction after 60 minutes in association with continued reduction in prostaglandin synthesis, it is clear nevertheless that significant vasoconstriction does occur soon after indomethacin administration. As the hemodynamic responses do not correspond to the changes in prostaglandin concentrations, it appears likely that this vasoconstriction involves more than simply a change in the synthesis of vasodilating prostaglandins and implies the action of one or more vasoconstricting substances. It may be that, in the basal state, the influence of vasodilating prostaglandins is balanced by vasoconstrictor hormones in the circulation or from sympathetic nerves. The rapid reduction of vasodilating prostaglandins would then leave these vasoconstrictors unopposed, leading to an observed response of overall vasoconstriction. However, these vasoconstrictors are likely under feedback control and their subsequent reduction, perhaps somewhat delayed, would allow organ perfusion to return gradually to its basal level. Although the exact nature of the transient vasoconstriction observed after indomethacin is not clear, the data presented in Fig. 7 are suggestive that it could involve the action of angiotensin II. As seen in Fig. 7, *B*, the systemic infusion of angiotensin II results in a pattern of uterine and systemic responses strikingly similar to that observed with indomethacin. It is notable that we have not observed this phenomenon, that is, delayed vasoconstriction in the uterine vasculature producing a biphasic response in uteroplacental blood flow, in response to other vasoconstrictors; infused norepinephrine,[27] epinephrine,[28] and vasopressin (recent observations) caused only a fall in uteroplacental blood flow, indicating no delay in the uterine vascular response to these hormones. It may also be noted that McLaughlin et al.[11] observed no effect of prior α-receptor blockade on the early responses to indomethacin, also making a catecholamine-mediated mechanism unlikely.

There are several ways in which vasoconstriction due to an apparent increase in angiotensin II–like activity could be mediated. First, angiotensin II appears to be having a tonic vasoconstrictor effect on the systemic vasculature in pregnant sheep, in that mean arterial pressure falls when saralasin is administered.[29, 30] A reduction of the opposing action of vasodilating prostaglandins by indomethacin could then leave endogenous angiotensin II unopposed, resulting in generalized vasoconstriction. Furthermore, although indomethacin is thought to reduce production of renin by a direct action on the kidney,[31] the decreases in plasma renin activity observed in the present study are also consistent with a negative feedback mechanism adjusting circulating angiotensin II concentrations to a lower level appropriate to balance the reduced level of prostaglandin synthesis.

Although indomethacin is known to inhibit prostaglandin synthesis, this compound is also known to have many other actions.[12, 32] It is possible, therefore, that indomethacin might induce vasoconstriction by direct stimulation of a vasoconstrictor, rather than indirectly through reduction of vasodilating prostaglandins. For example, indomethacin might inhibit angiotensinase, thereby increasing the circulating levels of angiotensin II. Alternatively, as lipoxygenase is not inhibited by indomethacin,[12] cyclo-oxygenase inhibition could result in diversion of increased amounts of arachidonic acid into the lipoxygenase metabolic pathway, several components of which (for instance, leukotrienes) have vasoconstrictor properties. In these situations, the reduction in prostaglandin levels could represent an epiphenomenon of the hemodynamic response to indomethacin, that is, the inhibition of prostaglandin synthesis may not be causally related to the observed vasoconstriction. Other evidence has been presented recently which supports this suggestion of a more direct action of indomethacin; in these studies,[33] indomethacin produced acute, severe vasoconstriction, whereas large doses of another prostaglandin synthetase inhibitor, ibuprofen, had no such effect. Furthermore, prior treatment with ibuprofen, presumably suppressing prostaglandin synthesis, did not inhibit the vasoconstrictor effect of indomethacin. Thus the mechanism of the vasoconstriction that follows indomethacin appears to be more complex than the simple reduction of vasodilating prostaglandins and likely involves one or more vasoconstrictor compounds. Further studies are planned to investigate this response to indomethacin in more detail.

We have concluded from the present study that, in the unstressed pregnant sheep, uteroplacental blood flow is not directly related to the level of prostaglandin synthesis, as reflected by plasma prostaglandin concentrations, and that uteroplacental perfusion can be maintained at normal levels despite substantial reductions in prostaglandin production. Data from other studies are suggestive that vasodilating prostaglandins may be more important in the maintenance of uteroplacental perfusion under abnormal or "stress" circumstances. However, our present observations raise a question about the interpretation of studies involving the use of indomethacin. Although its action of inhibiting prostaglandin synthesis is well established, it cannot be assumed that this is the only action which indomethacin has in an experimental situation. Before conclusions regarding the role of endogenous prostaglandins can be made with any confidence from such studies, it will be important to characterize further the mechanism of the vasoconstriction which follows indomethacin administration. In particular, it will be essential to determine whether the hemodynamic responses are indeed due to prostaglandin inhibition or whether indomethacin induces vasoconstriction through other, more direct mechanisms unrelated to changes in prostaglandin synthesis.

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Platelet activation in preeclampsia

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Platelet activation was assessed in hospitalized third-trimester patients with preeclampsia ($n = 11$) or chronic hypertension with superimposed preeclampsia ($n = 11$) and in healthy outpatient pregnant controls ($n = 10$) by measuring plasma β-thromboglobulin, platelet factor 4, the platelet aggregate ratio, and the amount of collagen required to produce half-maximal aggregation velocity (K_d). Only plasma β-thromboglobulin levels differed significantly between patients with preeclampsia (50.1 ± 37.9; $p < 0.05$) or chronic hypertension with superimposed preeclampsia (47.6 ± 16.3; $p < 0.01$) and the control subjects (22.5 ± 11.3). β-Thromboglobulin values in patients with preeclampsia, but not chronic hypertension with superimposed preeclampsia, correlated directly with 24-hour urinary protein loss ($r = 0.93$, $p < 0.001$) and serum creatinine levels ($r = 0.62$, $p < 0.05$) and inversely with creatinine clearance ($r = 0.60$, $p = 0.05$). We conclude that (1) β-thromboglobulin is elevated in patients with preeclampsia or chronic hypertension with superimposed preeclampsia, (2) the normal platelet aggregate ratio and the K_d indicate that the increase in β-thromboglobulin is not due to an intrinsic change in platelet responsiveness, and (3) the elevation of β-thromboglobulin in patients with either preeclampsia or chronic hypertension with superimposed preeclampsia appears to be secondary to platelet consumption in the microvasculature, although in patients with preeclampsia altered renal function may be contributory. (AM J OBSTET GYNECOL 1985;151:494-7.)

Key words: Preeclampsia, β-thromboglobulin, platelet factor, proteinuria

Preeclampsia is associated with vasospasm, occlusive vascular lesions within numerous organ systems including the placental bed, and activation of the coagulation system. Evidence for involvement of circulating platelets in preeclampsia includes a reduction in platelet count,[1-4] a change in platelet aggregability,[5] and raised plasma levels of the platelet-specific protein β-thromboglobulin.[6-8] Possible explanations for the increased platelet activation are an intrinsic change in platelet responsiveness or increased consumption and turnover within the microvasculature secondary to hypertensive vasospasm.

To examine these issues, we measured plasma β-thromboglobulin, platelet factor 4, the platelet aggregate ratio, and the amount of collagen required to produce half-maximal aggregation velocity (K_d). β-Thromboglobulin and platelet factor 4 are platelet-specific proteins that reflect platelet aggregation and degranulation. The platelet aggregate ratio measures circulating platelet aggregates, and the K_d estimates intrinsic platelet responsiveness. In addition, platelet activation was compared to other parameters of the preeclamptic syndrome.

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Table I. Patient profile

	Patients with "pure" preeclampsia	*Patients with chronic hypertension with superimposed preeclampsia*	*Control subjects*
No. of women	11	11	10
Maternal age (yr)	26.2 ± 6.2	27.7 ± 5.8	29.1 ± 3.2
Gestational age at delivery (wk)	36.9 ± 2.9	35.6 ± 3.1	39.3 ± 0.9
Nulliparous (%)	100	27	40
Mean arterial blood pressure (mm Hg)*	99.2 ± 8.0	116.9 ± 12.1	81.7 ± 7.1
Uric acid (mg/dl)	6.6 ± 0.8	6.8 ± 1.7	—
24 hr urinary protein (gm)	3.6 ± 4.3	5.4 ± 5.7	—
Creatinine clearance (ml/min)	92.5 ± 15.3	123.7 ± 30.8	—
Platelet count (× 10^3)	165 ± 53	184 ± 49	212 ± 59
Platelets <150,000 (%)	36	36	10

Values given are means ± 1 SD.

*Peak mean arterial blood pressure on the day platelet activation studies were drawn.

Table II. Platelet activation in hypertensive versus normotensive pregnant patients

	n	*β-Thromboglobulin (ng/ml)*	*Platelet factor 4 (ng/ml)*	*Platelet aggregate ratio*	*Kd* (μg/ml)*
Patients with "pure" preeclampsia	11	50.1 ± 37.9†	6.2 ± 7.2	0.91 ± 0.06	0.42 ± 0.42
Chronic hypertension with superimposed preeclampsia	11	47.6 ± 16.3‡	4.7 ± 2.4	0.88 ± 0.07	0.50 ± 0.25
Control subjects	10	22.5 ± 11.3	3.2 ± 3.8	0.90 ± 0.10	0.38 ± 0.36

Values given are means ± 1 SD.

*K_d, Amount of collagen required to produce half maximal aggregation velocity.

†$p < 0.05$ when compared to values for control subjects.

‡$p < 0.01$ when compared to values for control subjects.

Material and methods

Platelet activation was assessed in 22 hospitalized third-trimester preeclamptic patients within 10 days of delivery. Preeclampsia was defined by the standard criteria of blood pressure, proteinuria, edema, and central nervous system signs.[9] If there was either a prepregnancy history of hypertension (treated or not) or a documented elevation of mean arterial blood pressure during the first (95 mm Hg) or early second (90 mm Hg) trimesters, the patient was classified as chronically hypertensive with superimposed preeclampsia ($n = 11$). Patients without evidence of chronic hypertension were classified as having "pure" preeclampsia ($n = 11$). Ten healthy outpatients between 34 and 38 weeks' gestation served as the control subjects. Demographic information for the three groups is listed in Table I.

Blood was obtained by clean venipuncture using a 21-gauge butterfly without backpressure. Plasma β-thromboglobulin and platelet factor 4 were measured by radioimmunoassay with the use of commercially available kits (Amersham and Abbott Laboratories, respectively). The platelet aggregate ratio was performed according to the method of Wu and Hoak.[10] The amount of collagen required to produce half-maximal aggregation velocity (K_d) was measured as described previously by Rossi and Louis.[11]

Additional blood parameters assessed in the hospitalized patients within 24 hours of the platelet studies were platelet count, hemoglobin/hematocrit, uric acid, creatinine, urea nitrogen, total protein, and albumin. Twenty-four hour urine collections were used to quantitate protein loss and measure the creatinine clearance. Blood pressure was measured every 4 hours in the left lateral decubitus position, and the mean arterial blood pressure was calculated according to the following formula:

$$\text{MAP} = \frac{\text{Systolic blood pressure} + (2 \times \text{diastolic blood pressure})}{3}$$

Gestational age was determined by the best fit of menstrual dates, ultrasound results if available, and neonatal Dubowitz examination. Birth weights were compared to the chart of Brenner et al.[12] for newborn infants at sea level. A neonate was considered to be small for gestational age if his or her birth weight was less than or equal to the tenth percentile.

Statistical analysis was done by Student's *t* test or calculation of correlation coefficient (r) performed ac-

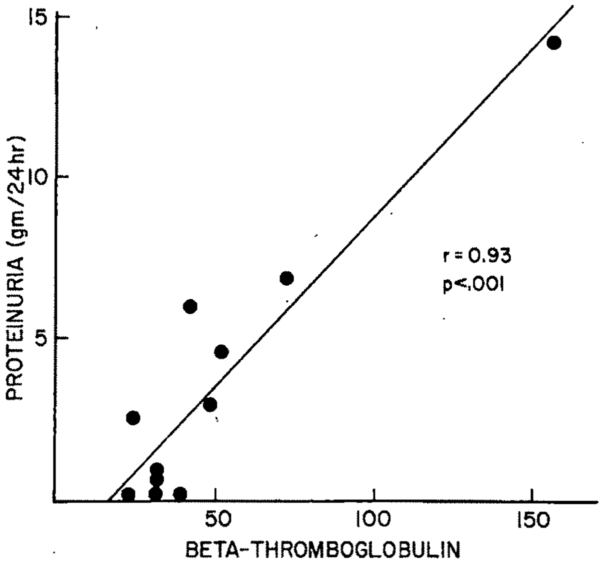

Fig. 1. In the group of patients with "pure" preeclampsia there was a significant relationship between β-thromboglobulin levels and 24-hour urinary protein loss (r = 0.93, $p < 0.001$).

cording to the method of least squares. Significance was assumed at $p < 0.05$.

Results

The mean values (±1 SD) of β-thromboglobulin, platelet factor 4, platelet aggregate ratio, and K_d for the three groups are listed in Table II. Only β-thromboglobulin differed significantly between the control patients and those with preeclampsia ($p < 0.05$) or chronic hypertension with superimposed preeclampsia ($p < 0.01$). β-Thromboglobulin and platelet factor 4 measurements were closely related (r = 0.85; $p < 0.001$), but the latter were not statistically different from the control patients.

In the preeclampsia group β-thromboglobulin correlated directly with 24-hour urinary protein loss (r = 0.93, $p < 0.001$; Fig. 1) and serum creatinine levels (r = 0.62, $p < 0.05$) and inversely with creatinine clearance (r = 0.60, p = 0.05). These correlations were not present in the group with chronic hypertension with superimposed preeclampsia. There was no apparent relationship between β-thromboglobulin and mean arterial blood pressure in either group regardless of whether the highest or average daily mean arterial blood pressure was utilized. Similarly, there were no identifiable relationships between β-thromboglobulin and any of the other maternal clinical parameters.

Each control patient delivered an appropriate–for–gestational age infant. Forty-five percent (5/11) of the neonates of mothers with preeclampsia and 36% (4/11) of the neonates of mothers with chronic hypertension with superimposed preeclampsia were small for gestational age. However, β-thromboglobulin was not predictive of small–for–gestational age infants in either group (Fig. 2).

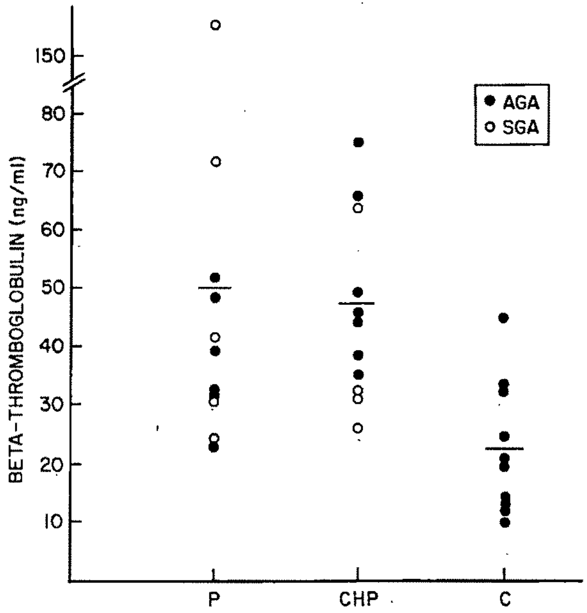

Fig. 2. This histogram displays the individual β-thromboglobulin measurements in those patients with "pure" preeclampsia (*P*, n = 11) or chronic hypertension with superimposed preeclampsia (*CHP*, n = 11) compared to controls (*C*, n = 10). β-Thromboglobulin levels were not predictive of small–for–gestational age newborn infants *(open circles)*.

Comment

Plasma β-thromboglobulin levels in hospitalized third-trimester patients with either preeclampsia or chronic hypertension with superimposed preeclampsia were elevated when compared to levels in healthy outpatient pregnant control subjects. Plasma platelet factor 4 values were closely related to the β-thromboglobulin measurements, but the elevations of platelet factor 4 in the hypertensive patients were not statistically significant. One explanation for the latter finding is the relatively wide standard deviations observed. Another possibility is the difference in half-life of β-thromboglobulin and platelet factor 4. β-Thromboglobulin and platelet factor 4 are platelet-specific proteins that are released when platelets are aggregated by various agents, including thrombin, adenosine disphosphate, adrenalin, and collagen.[13] Although the two proteins are markedly similar in structure, the half-life of β-thromboglobulin is about 100 minutes whereas platelet factor 4 is cleared so rapidly that its half-life cannot be accurately estimated.[14] Both β-thromboglobulin and platelet factor 4 are cleared by the renal glomeruli, but the rapid disappearance of platelet factor 4 suggests that it may be removed initially by a simple process such as adsorption to endothelial cells.[14]

The platelet aggregate ratio and K_d were similar in the three groups of patients. These findings indicate that any increase in platelet activation in the patients with preeclampsia or chronic hypertension with superimposed preeclampsia was not secondary to an intrinsic change in platelet responsiveness. Rather, as postulated by other authors,[5-8] a more likely explanation for the rise in β-thromboglobulin was increased platelet turnover because of aggregation stimulated by hypertensive vasospasm and the concomitant endothelial damage. Our inability to identify a relationship between β-thromboglobulin and the degree of hypertension does not preclude increased platelet turnover within the microcirculation.

β-Thromboglobulin correlated directly with urinary protein loss and serum creatinine, and inversely with creatinine clearance, in patients with preeclampsia. There were no identifiable relationships between β-thromboglobulin and renal function in patients with chronic hypertension with superimposed preeclampsia. Further, β-thromboglobulin levels did not correlate with the other maternal parameters or fetal growth in either group. These observations suggest that altered renal function contributes to the rise in β-thromboglobulin in patients with preeclampsia. The mechanism involved could be a decrease in renal clearance of β-thromboglobulin or an increase in fibrin deposition and platelet aggregation within the renal glomeruli.

The criteria used for separating patients into preeclampsia and chronic hypertension with superimposed preeclampsia groups were chosen in an attempt to identify as "pure" a group as possible. It is therefore more likely that if a misclassification happened, it was by placing a preeclampsia patient into the group with chronic hypertension with superimposed preeclampsia rather than vice versa. Within this limitation, the observations in this study indicate that β-thromboglobulin is elevated but the platelet aggregate ratio and K_d are normal in patients with preeclampsia or chronic hypertension with superimposed preeclampsia. Platelet consumption in the microvasculature associated with hypertensive vasospasm appears to be an important etiologic factor for the rise in β-thromboglobulin in both groups, although in preeclampsia patients altered renal function may be contributory.

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Fetal heart rate response to maternal exercise

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Twenty-five pregnant women without complications underwent fetal heart rate evaluation during a program involving exercise at a relative intensity of 61% to 73% of maximal capacity during the course of the pregnancy. Assessment of the influence of gestational age on the fetal heart rate response to exercise and evaluation of the course of fetal heart rate recovery were the main goals of the study. The results of the study confirmed previous findings that fetal heart rate is accelerated after maternal exercise. However, contrary to other studies we found no effect of gestational age on the fetal heart rate response to exercise. Neonatal findings provided further evidence that quantitated maternal exercise up to 70% of maximal capacity does not interfere with normal fetal growth and development. (Am J Obstet Gynecol 1985;151: 498-501.)

Key words: Fetal heart rate, maternal exercise, pregnancy

Since participation by pregnant women in exercise and fitness programs has become more customary, considerable interest in the fetal heart rate response during such exercise has developed. Dressendorfer and Goodlin[1] observed an acceleration in fetal heart rate during progressive cycle ergometry exercise during the third trimester in pregnant women who had been regularly participating in lap swimming. Hauth et al.[2] performed nonstress tests during the third trimester in pregnant women who jogged 1.5 miles three times per week. All tests before and after jogging were reactive. The fetal heart rate before jogging ranged from 140 to 150 bpm, and the fetal heart rate after jogging ranged from 180 to 204 bpm. Fetal heart rate returned to levels before jogging in an average of 22 minutes. Pernoll et al.[3] showed a difference between the fetal heart rate response to mild exercise in women of <35 weeks' gestation and women of >35 weeks' gestation. In a previous study in our laboratory[4] we observed a fetal heart rate acceleration following 25 minutes of aerobic exercise. However, we did not evaluate the influence of gestational age. In addition, our data were based on auscultation of fetal heart rate for a 15-second period with the use of a Doppler ultrasonic instrument (Doptone).

The present study was designed to further examine fetal heart rate responses during maternal aerobic exercise. Specific design features of this study included (1) precise quantification of maternal exercise intensity and duration, (2) utilization of continuous fetal heart rate monitoring, (3) assessment of the influence of gestational age on the fetal heart rate response, and (4) evaluation of the course of fetal heart rate recovery from maternal exercise.

Material and methods

Twenty-five healthy pregnant women with no complications participated in the study. Informed consents were obtained from all women. The subjects were aged 25 to 33 years and 24 of the women were primiparous. All women had been regular participants in a three-times-weekly supervised aerobic exercise program since early in their pregnancies (range, 13 to 18 weeks with an average of 14.5 weeks' gestation) as part of a larger study on maternal exercise.

Fetal heart rate tracings were obtained for each woman on alternate weeks beginning at 28 weeks' gestation and continuing until 38 weeks' gestation. Thus an attempt was made to obtain six serial tracings on each woman. Poor or inadequate tracings, delivery before 38 weeks' gestation, and vacation periods reduced the number of available tracings from 150 to 121. All tracings were obtained with a Corometrics model 111 fetal monitor. At each study session a 10-minute tracing of fetal heart rate was recorded before the exercise began. Within 1 minute following the exercise, another 10-minute tracing was obtained. A mid-range value was determined for each 1 minute segment of the 10-minute fetal heart rate tracings before and after exercise. With the use of these 10 mid-range values, a "mean" mid-range value before and after exercise was calculated, and this value was defined as the baseline fetal heart rate.

The exercise sessions consisted of 10 minutes of warm-up flexibility activity followed by 30 minutes of continuous aerobic activity. Walking, jogging, and stationary cycling were the modes of aerobic activity. Exercise was individually prescribed for each woman based on exercise test results and according to methods

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Table I. Baseline fetal heart rate values (bpm ± SD) before and after exercise, according to weeks of gestation (n = 11)

	Week of gestation				
	28	*32*	*34*	*36*	*38*
Before exercise	141 ± 6	140 ± 6	140 ± 7	142 ± 6	138 ± 11
After exercise	150 ± 9	147 ± 7	149 ± 10	153 ± 7	148 ± 9

Table II. Fetal heart rate values in beats per minute for the final minute before exercise and each minute after exercise (n = 25)

Relationship of minute to exercise	*Mean (bpm)*	*Range (bpm)*
10th before	140	126-154
1st after	153*	138-171
2nd after	151*	135-167
3rd after	151*	135-166
4th after	149*	134-164
5th after	148*	132-163
6th after	147*	133-160
7th after	147*	134-159
8th after	147*	135-158
9th after	146*	135-158
10th after	145*	130-157

*Significantly different from 10th minute before exercise ($p < 0.01$).

Table III. Clinical classification of fetal heart rate responses before and after exercise

	Normal range (120-160 bpm)		*Moderate tachycardia (161-180 bpm)*	
	%	*No. of tracings*	%	*No. of tracings*
Before exercise	99.2	120/121	0.8	1/121
After exercise	90.1	109/121	9.9	12/121

described in a previous publication.[4] The relative exercise intensity averaged 68.5% (range, 60.8% to 73.1%) of maximal aerobic capacity. The resultant metabolic cost of the activity expressed as the ratio of estimated exercise metabolic rate to estimated resting metabolic rate averaged 6.8 (range, 3.9 to 10.6). Maternal heart rates averaged 148 bpm (range, 132 to 156 bpm). This exercise regimen is consistent with the American College of Sports Medicine guidelines for developing and maintaining fitness in healthy adults[5] and is comparable to typical community-based fitness or aerobic training programs.

The data were analyzed by means of analysis of variance with repeated measures.[6] When a significant F ratio was determined, post hoc comparisons were performed according to Dunnett's test, and pooled standard errors of the means were calculated[6] (p values <0.01 were taken to indicate statistical significance).

Results

Eleven of the subjects had complete serial data for gestational weeks 28, 32, 34, 36, and 38. These data were analyzed separately to evaluate the effect of gestational age on the fetal heart rate response to exercise. Table I shows mean preexercise and postexercise baseline fetal heart rate as a function of gestational age. There were no significant differences between weeks of gestation in the fetal heart rate response to exercise ($F = 1.33$, $p = 0.28$).

All values from these 11 subjects plus the remaining 14 subjects were then averaged and the values before and after exercise were compared. The baseline fetal heart rate after exercise was significantly greater than the fetal heart rate before exercise (before: 140, after: 149; $F = 56.53$; $p < 0.001$; SEM, 2.86).

Fetal heart rate differences before and after exercise were further examined by a comparison of mid-range values for the final minute before exercise (tenth minute) with fetal heart rate values for each minute after exercise. The overall F value was significant, and post hoc comparisons were performed (overall $F = 37.21$; $p < 0.001$; SEM = 3.02). These comparisons are shown in Table II. Mid-range fetal heart rate values for each minute after exercise were significantly greater than the final value before exercise. Fetal heart rate declined at a rate of approximately 1.8 bpm over the 10-minute recovery period after exercise.

Table III demonstrates the clinical classification of baseline fetal heart rate response before and after exercise in the 121 tracings obtained in the 25 patients. Less than 1% of the responses before exercise were found to be in the moderate tachycardia range of 161-180 bpm. In contrast, almost 10% of the responses after exercise were found to be in the moderate tachycardia range. There were no cases of marked tachycardia (≥180 bpm) or bradycardia (≤120 bpm) either before or after exercise.

Four individual case examples are shown in Fig. 1. Case 1 was a 32-year-old primigravid woman at 36 weeks' gestation. Mean baseline fetal heart rate was 151 bpm during the tracing before exercise and 155 bpm during the tracing after exercise. Case 2 was a 30-year-

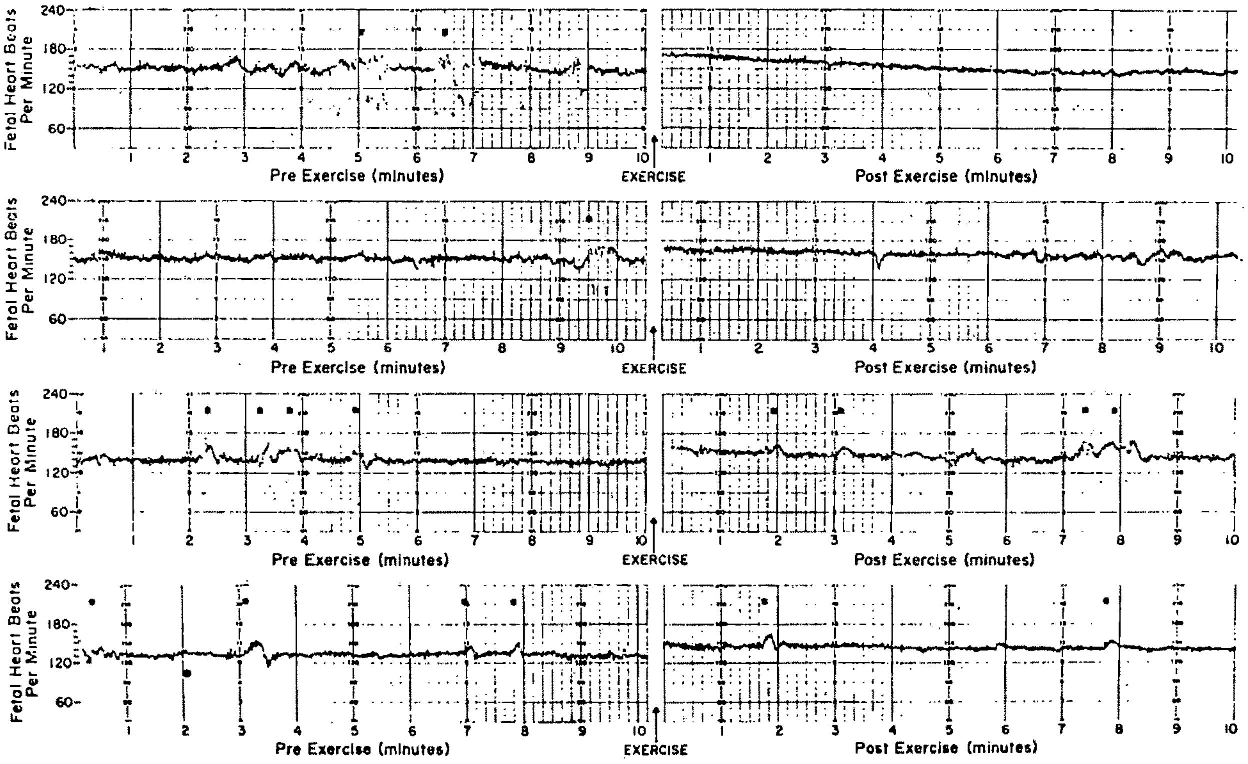

Fig. 1. Fetal heart rate recovery after exercise.

old multigravid woman at 36 weeks' gestation. The baseline fetal heart rate before and after exercise was 152 and 159 bpm, respectively. Case 3 was a 26-year-old primigravid woman at 34 weeks' gestation. Baseline fetal heart rate was 146 bpm and after exercise the fetal heart rate was 146 bpm. Case 4 was the same 26-year-old woman at 36 weeks' gestation. Baseline fetal heart rate before and after exercise averaged 132 and 142 bpm respectively. In each case the tracing illustrates the fetal heart rate 10-minute recovery pattern after exercise.

Twenty-four of the women delivered normal infants with a mean birth weight of 3533 gm (range, 2963 to 4338 gm) and a 5-minute Apgar score of 9 (range, 8 to 10). Mean duration of pregnancy was 40.2 weeks (range, 36 to 42 weeks). One subject delivered a 3062 gm infant with 1- and 5-minute Apgar scores of 1 and 3, respectively. This infant was post term and suffered severe perinatal asphyxia. The infant died 1 week later. No fetal heart rate abnormalities had been noted in this infant during the study period.

Comment

The results of the present study confirm our earlier findings[4] that maternal exercise is associated with an acceleration of fetal heart rate. Using periodic 15-second auscultation of fetal heart rate by Doptone in our earlier work, we found a 4 bpm acceleration after 20 minutes of cycling exercise and a 3 bpm elevation after 5 minutes of recovery. In the present study a somewhat more pronounced fetal heart rate response was found: fetal heart rate was elevated by 13 bpm immediately after exercise and was elevated by approximately 8 bpm after 5 minutes of recovery. This study differs from our earlier work by the addition of brisk walking and jogging as exercise modes, continuous fetal heart rate monitoring, and a total duration of exercise that was 5 minutes longer. The more pronounced fetal heart rate acceleration in the present study may be related to any of these three differences.

Our results also support those of Dressendorfer and Goodlin,[1] who reported a 7 bpm increase in fetal heart rate during progressive cycle ergometry exercise testing in trained pregnant women, and those of Hauth et al.[2] However, Hauth et al. found that fetal heart rate was >180 bpm in 60% of their responses after jogging and >170 bpm in 73% of their responses after jogging. In contrast, we found no instances of fetal heart rate >180 bpm, and only 10% of responses in our study were in the range of 161 to 180 bpm. Differences in study design may account for these differences. Exercise in the present study was individually prescribed according to estimated maximal aerobic capacity. Because of individual differences in maximal aerobic capacity, the resultant absolute exercise intensities ranged from 3.9 to 10.6 times resting metabolic rate. In spite of this large range in absolute exercise intensities, the relative exercise intensity was within a small range (60.8% to 73.1%) of maximal aerobic capacity. Thus the exercise was standardized according to relative exercise intensity. Subjects in the study of Hauth et al. were exercising at absolute intensities of approximately 10.3 to 12.2 times resting metabolic rate (calculating from jogging speeds of 6.25 to 7.5 mph). Since they did not perform exercise tests to evaluate maximal aerobic capacity, the relative exercise stress for each individual subject is not known. Though it cannot be stated with certainty, we suspect that their subjects were also exercising at higher relative intensities than our subjects. In addition, it is not clear from their data whether the fetal heart rates after jogging were accelerations subsequent to fetal movement or whether they represented a baseline fetal heart response before and after exercise.

Pernoll et al.[3] found that fetal heart rate declined after exercise in women of <25 weeks' gestation compared to a fetal heart rate increase in women >35 weeks' gestation. In contrast to their findings, we found no effect of gestational age on the fetal heart rate response during maternal exercise. The work of Pernoll et al. was based on 6 minutes of mild bicycle exercise whereas the exercise in our study consisted of moderate level walking, jogging, and cycling for 30 minutes. Another difference between our studies may be the result of the population characteristics. Their data are based on a population of both normal and mildly abnormal women whereas our data were obtained from women with no obstetric or medical complications. The differing nature of our exercise or study populations probably accounts for the different results obtained.

In our earlier study[4] we discussed factors that may be related to the acceleration of fetal heart rate following maternal exercise. These factors included fetal arousal, placental transfer of elevated maternal catecholamines, an increase in maternal and fetal temperature, or a reduction in uterine blood flow. It has also been suggested[2, 7] that fetal tachycardia after exercise may be a compensatory response to undetected bradycardia *during* maternal exercise. In the early stages of our research, we attempted to record fetal heart rate during exercise. This included fetal heart rate monitoring while the women were cycling in an upright sitting posture and while cycling in a recumbent or semirecumbent position on an exercise table (Engineering Dynamics Corporation). In all cases we concluded that substantial artifact produced by leg and trunk motion rendered the tracings inadequate. However, in many instances we were able to count fetal heart rate during exercise by using the amplification system of the monitor. It was our observation that fetal heart rate during exercise was within the normal or moderate tachycardia range. We could not find any evidence of fetal bradycardia.

Finally, the neonatal findings at birth in this group of women confirm our previous findings and provide further evidence that quantitated maternal exercise up to 70% maximal capacity does not interfere with normal fetal growth and development.

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Umbilical artery velocity waveforms and intrauterine growth retardation

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A continuous-wave Doppler unit was used to obtain umbilical artery velocity waveforms and to calculate the peak-systolic/diastolic ratio, a reflection of vascular resistance distal to the point of measurement. A total of 587 examinations were performed on 189 women between 18 to 42 weeks' gestation. The neonates were divided in four groups based on their centile birth weight: <25%, 25% to 50%, 51% to 75%, and >75% for that gestational age. In all four groups the placental vascular resistance as reflected by the systolic/diastolic ratio fell progressively from the 24 weeks onward. In the lower weight group, placental vascular resistance between 31 to 39 weeks' gestation was higher than in the other three groups ($p < 0.05$). For measurements taken between 31 and 39 weeks' gestation we calculated the predictive value of this test in identifying the small–for–gestational age fetus. A ratio of ≥3 was defined as abnormal. For the entire population tested, sensitivity was 78% and the specificity was 83%. The predictive value of a positive test was 49%, but when hypertension was present, it was 66%. These findings may prove useful in identifying and managing pregnancies at risk for intrauterine growth retardation. (Am J Obstet Gynecol 1985;151:502-5.)

Key words: Doppler unit, umbilical artery, growth retardation

Although there are multiple causes of intrauterine growth retardation, the majority of cases are probably secondary to a diminution in the supply of nutrients and oxygen to the fetus.[1, 2] Clinical understanding of this disorder has been hampered by the paucity of techniques to measure placental function, the critical organ through which the transfer of nutrients occurs. Recently new technology has become available which provides the means to serially examine the development of the umbilical and uterine circulation during human pregnancy.[3]

Doppler ultrasound has been applied to the study of the circulation for the past decade. Proven usefulness has occurred in the study of peripheral vascular disease and the evaluation of carotid artery stenosis.[4, 5] Continuous-wave Doppler instruments are used routinely in most obstetricians' offices to provide evidence of fetal life by detecting aortic, umbilical, or cardiac signals. When these signals are summated by the technique of spectrum analysis, a pattern is created, the velocity waveform.[5] In this report we will describe in detail our experiences with 24 small–for–gestational age fetuses in whom umbilical artery velocity waveform studies were carried out in the last 11 weeks of pregnancy. This new procedure appears promising for diagnosis and offers a method of making an earlier detection and of developing treatment protocols.

Material and methods

The study group consisted of 189 women followed in the antepartum clinics of the Bronx Municipal Hospital Center and the Nassau Hospital. Women selected for study were those who had simultaneous nonstress testing, attended a high-risk pregnancy clinic, were referred because of suspicion of intrauterine growth retardation, or had volunteered for a routine screening program (Table I). Since the entire population was not examined, our data cannot represent the frequency in which an abnormal finding might occur. The project was approved by our clinical human investigation committees and signed informed consent was obtained upon entry into the study. A continuous-wave Doppler unit with a 4 MHz transducer and a power density of 6 mW/cm^2 was used to identify the umbilical artery velocity waveforms. A detailed review of the equipment, the methodology, and the theoretical consideration for practical use of velocity waveforms has been described previously.[3]

To describe briefly, with the woman in a supine, slightly tilted position, the Doppler transducer is placed on the abdomen and moved around until a clear signal is obtained (Fig. 1). In most instances, after 24 weeks'

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Table I. Study group characteristics

	No. of women	*%*
At risk	90	47
Hypertension	63	
Suspected intrauterine growth retardation*	47	
Poor obstetric history	8	
Medical complications	5	
Other factors	2	
No risk factors	99	53

*Some also had hypertension.

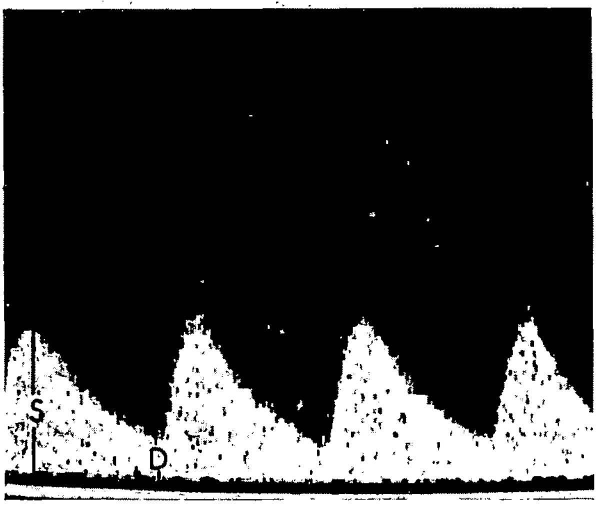


Fig. 1. Signal produced by Doppler transducer. *S* = Systolic; *D* = diastolic.

gestation the reading is easily obtained in the periumbilical area within 4 to 10 minutes. Once this signal is obtained, the image is frozen and the systolic/diastolic ratio is calculated (Fig. 2). These waveforms are quite characteristic and can easily be distinguished from maternal waveforms (based on rate) or the other fetal waveforms commonly seen, fetal aorta or fetal heart.[3] Four measurements of four separately imaged waveforms were taken during each session, and a mean value was calculated. In an earlier publication we reported on 89 women with appropriate–for–gestational age fetuses and 16 with intrauterine growth retardation. This report adds additional cases to amplify the data and to examine the intrauterine growth retardation group more closely.

Gestational age was determined by a combination of historical or clinical assessment, and ultrasound when available. The data was analyzed in three additional ways from that previously published. Regression curves were developed based on weight for gestational age quartiles. The Battaglia-Lubchencho graphs were used as the reference bases. Distribution columns were constructed to demonstrate range and means of weight quartiles and the lower-tenth percentile. Finally, sensitivity, specificity, and predictive values for positive tests were calculated.

Results

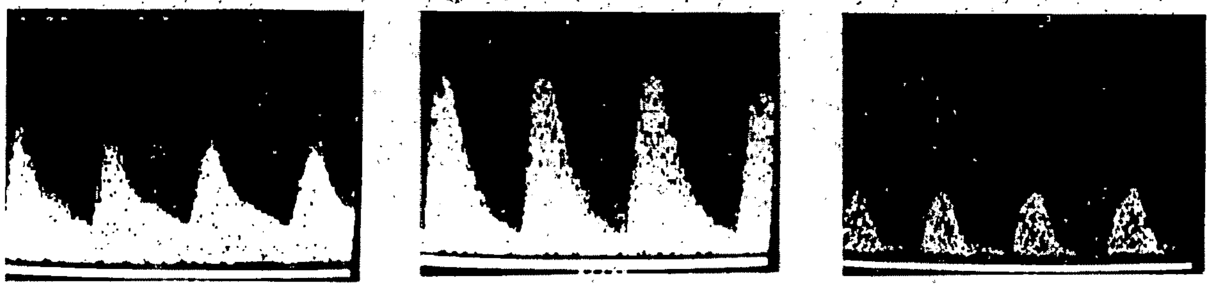
Previously it was noted that the average error encountered between consecutive examinations was 6% with a standard deviation of 5%. Hence, the maximum error in serial determinations is 16%. Only women having at least two measurements between 18 to 42 weeks' gestation were included in the analysis, except for two of the intrauterine growth retardation fetuses. Linear regression analysis for appropriate–for–gestational age fetuses revealed a standard deviation of 0.45 and a standard error of 0.002. For small–for–gestational age fetuses the standard deviation per gestational week is larger, 0.63, because values can range from 2.5 to 7. Values above 6 are not included.

The neonates were divided into four groups based on their quartile birth weight for sex and gestational age: group 1, >75%; group 2, 51% to 75%; group 3, 25% to 50%; group 4, <25%. The systolic/diastolic ratio was then plotted against gestational age for each individual group.

A total of 587 examinations were performed on 189 women. The results in the four groups are shown in Fig. 3. The data for the three upper weight groups are indistinguishable, but the ratios in the <25% group were significantly higher from 30 weeks' gestation onward ($p < 0.05$).

Fig. 4 plots the distribution of average systolic/diastolic values for women who had at least two measurements between 31 and 39 weeks' gestation. In addition, the lower 10% were separated out to conform to the established definition of intrauterine growth retardation. A total of 138 women having 409 measurements at each gestational period was similar in all four groups. Based on the distribution in Fig. 4 and for clinical utility, a normal systolic/diastolic ratio was defined as <3. A significant difference ($p < 0.01$) was seen in the <25% and <10% groups.

Next, calculations were carried out to determine the specificity, sensitivity, and predictive value of the umbilical artery velocity waveform ratios. With abnormal defined as ≥ 3, standard calculations were made as illustrated in Table II. For these calculations the standard definition of small–for–gestational age was used, i.e., <10% of weight for the gestational age.[6] The predictive value of a positive test was 49% significantly better than the prevalence rate in this population, which was 16% ($p < 0.01$). The predictive value of a negative test was 95%. The hypertensive group was segregated, and then the same analysis was carried out. There were 52 women with at least two measurements. The predictive value of the test was now 66% ($p < 0.001$). The predictive value of a negative test was 94%. For the pop-



S/D = 2.5 S/D = 5.8 S/D = ∝

Fig. 2. Calculated systolic/diastolic ratios.

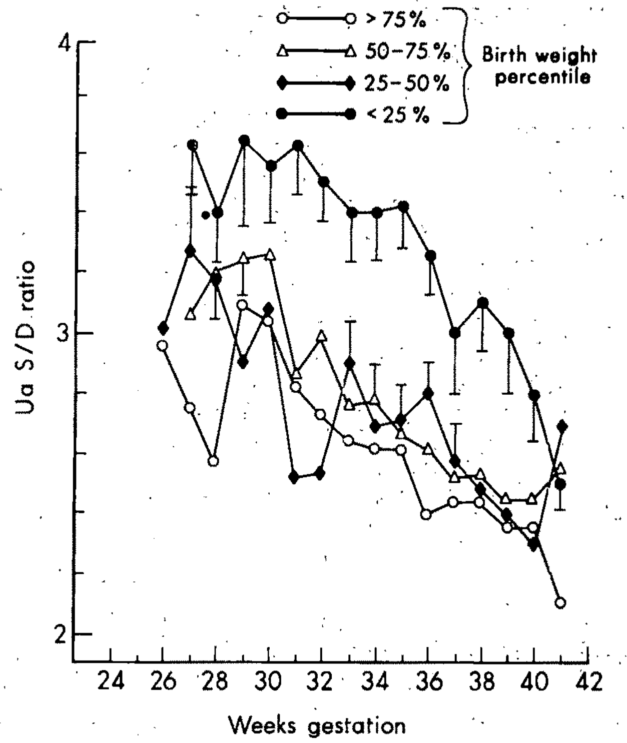


Fig. 3. Umbilical artery velocity ratios and birth weight quartiles.

Table II. Predictive values of systolic/diastolic ratios

	*Positive test**	*Negative test†*	*Total*
Total study group (n = 137)‡			
Small–for–gestational age	18	5	23
Average–for–gestational age	19	95	114
Total	37	100	137
Hypertensive patients (n = 52)§			
Small–for–gestational age	14	2	16
Average–for–gestational age	7	29	36
Total	21	31	52

*Mean systolic/diastolic ratio > 3 (31-39 weeks' gestation).

†Mean systolic/diastolic ratio < 3 (31-39 weeks' gestation).

‡Sensitivity, 78%; predictive value of positive test, 49%; specificity, 83%; predictive value of negative test, 95%.

§Sensitivity, 87%; predictive value of positive test, 66%; specificity, 81%; predictive value of negative test, 94%.

ulation as a whole, the sensitivity was 78% and the specificity was 83%.

Comment

Umbilical artery velocimetry appears to be a promising new technique for the study of fetal growth retardation. Although the classic definition of intrauterine growth retardation has been the lower-tenth percentile in weight for that gestational age, our data suggest that the twenty-fifth percentile may be a physiologic turning point. When umbilical artery velocity waveform systolic/diastolic ratios are calculated, both the twenty-fifth and tenth percentile groups were significantly higher than those of larger babies. It is possible that this may be a manifestation of the weight distribution of our population, but it may also reflect a deviation in the ratio that is reflective of a critical reduction in blood flow which impairs fetal growth.[2]

An abnormal test proved to have good predictability for intrauterine growth retardation. A positive test was predictive to the level of 49%, but if the woman had hypertension, it was 66%. This is superior or comparable to most reports utilizing morphologic ultrasound parameters. We have begun studying uterine artery velocimetry, and we suspect that the higher predictability in hypertensive women is because of the higher probability of increased uterine artery resistance.[7]

As in the case of fetal heart rate monitoring, the predictive value of a negative test is 95%. Thus a fetus with a normal umbilical artery velocity waveform pattern is very likely to be normal in size. It is not unexpected that abnormal patterns should not always be associated with a small fetus. Velocity is only a component of flow but not a measure of flow itself. Furthermore, the fetus has compensatory mechanisms for the uptake of nutrients when flow is diminished.

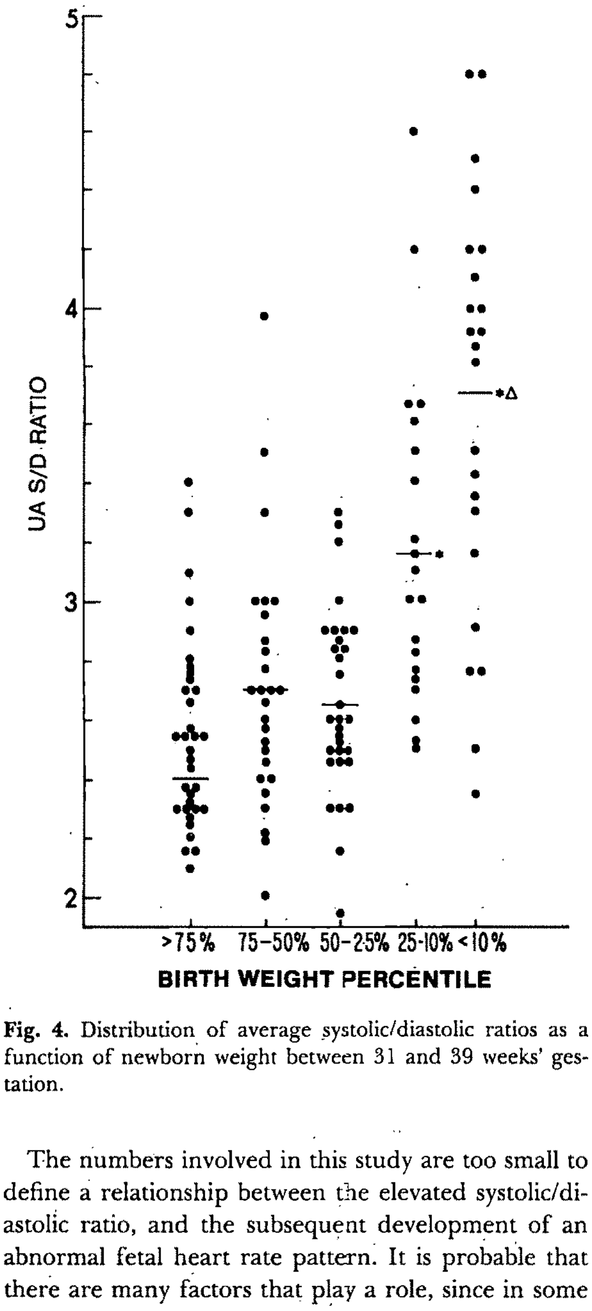


Fig. 4. Distribution of average systolic/diastolic ratios as a function of newborn weight between 31 and 39 weeks' gestation.

The numbers involved in this study are too small to define a relationship between the elevated systolic/diastolic ratio, and the subsequent development of an abnormal fetal heart rate pattern. It is probable that there are many factors that play a role, since in some cases the systolic/diastolic ratio may appear elevated as early as 16 weeks' gestation, whereas others develop as a secondary event after normal placentation had been established.

The measurement of a systolic/diastolic ratio as proposed in the study depends on pattern recognition of the vessel.[8] It is a reflection of resistance beyond the point of measurement, and therefore it is a crude indicator when compared to direct calculations of flow. However, in order to measure flow, the fetal vessel must be visualized, the diameter measured, and the angle of the transducer signal must be known.[9, 10] Since the fetus does not remain in a fixed position, these measurements can be quite time consuming, and with the combined imaging and pulsed Doppler transmission, the energy absorption or power density exceeds currently recommended levels. Those reports that have measured flow have suggested a decrease of approximately 25% below the expected value of 115 ml/kg/min when intrauterine growth retardation is present.[11] Gill et al.[12] measured umbilical venous flow in 11 patients with intrauterine growth retardation and had a positive predictive value of 62%. Their numbers are not significantly different from ours when their appropriate–for–gestational age numbers are brought to parity with ours, e.g., their corrected results would be 53%.

As shown in this report, accurate velocity waveforms can be obtained without imaging the fetus and umbilical cord. This approach makes the procedure simpler and significantly reduces ultrasound exposure. Finally, the simplicity allows repetitive examinations so that the method is of value as a screening test and may prove to be an important fetal surveillance tool.

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Estrogen stimulates formation of lamellar bodies and release of surfactant in the rat fetal lung

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Estrogen has been shown to enhance the β-adrenergic–receptor sites in rabbit lung tissue. Because of the possible relationship between β-adrenergic receptor and surfactant maturation, we studied the electron microscopic changes in type II cells of lung tissue of control as well as estrogen-treated developing rat fetuses. The amniotic sacs of 31 gravid rats, at day 15 to 21 of a 22-day pregnancy, were injected with either estradiol phosphate or saline solution or were not injected. The contralateral horn pregnancies acted as controls. In the noninjected and saline solution–injected controls multilamellar bodies started to be visible on day 18 and increased in number as well as size on days 18 and 19. On day 20, the process of release of the phospholipids into the alveolar cavities became apparent. After 48 hours of exposure to estradiol, there was a marked enhancement of the development of lamellar bodies, which were visible on day 17. The increase was both in number and in complexity. In the estradiol-injected animals, the release of the contents of the lamellar bodies into the alveolar cavity started 1 to 2 days earlier than in the control animals. There is an indication that propranolol injection decreases both the formation and the release of the phospholipids. We conclude that estrogen stimulates both the formation and the release of surface-active phospholipids in rat fetal lung tissue. (Am J Obstet Gynecol 1985;151:506-14.)

Key words: Fetal lung, estrogen, surfactant release, β-adrenergic receptor, phospholipids

It is well established that the synthesis of surfactant takes place in type II cells of the lung.[1] The developmental process, which starts late in fetal life, involves the formation and storage of these phospholipids in eosinophilic lamellar bodies, subsequently followed by exocytotic release into the alveolar space.[2] Both hormonal and nervous mechanisms are considered to be involved in the control of this process. Thus it has been shown that corticosteroids,[3,4] thyroxine,[5,6] prolactin,[7,8] and β-adrenergic agonists[9] enhance the maturation and/or release of surfactant. Cortisol treatment is also associated with an increase in the number of β-adrenergic receptors in the lung, an effect which might explain why corticosteroids are effective in the treatment of respiratory distress syndrome.[8] Recently, we have shown that estrogen has the very same stimulant effect on β-adrenergic–binding sites in the immature and mature rabbit lung in vivo, whereas progesterone is inhibitory in this respect.[10] Since the rat fetus near term is exposed to increasing concentrations of estrogen, concomitant with a fall in progesterone,[11] the present study was designed to elucidate further the action of estrogen on the development and release of surfactant into the alveolar space of rat fetuses in late gestation.

Material and methods

Thirty-one pregnant Sprague-Dawley white rats were used. The gestational ages of the fetuses were calculated from the day of mating with the total length of gestation being 22 ± 1 days. The animals were anesthetized with ether. The abdominal wall was opened, the uterine horns exposed, and the number of fetuses in each horn noted. Water-soluble estradiol phosphate, 4 μg in 10 μl, was injected into the amniotic sac of a selected fetus with a 100 μm pore–size glass microelectrode. The same procedure was done on one of the amniotic sacs in the opposite horn, with saline solution only injected to act as a placebo control. The horns were repositioned in the abdomen, and the abdominal wall was closed. At a second operation, performed either 24 or 48 hours later, the fetuses were removed and the lungs dissected free and prepared for electron microscopy, as described below. The fetuses removed had been injected with either estrogen or saline solution (placebo control) or were noninjected (pure control).

The experimental protocol was as follows.

Control experiments. Fourteen pregnant rats were put to death at various stages of gestation, between day 17 and day 22 (or day of delivery). These experiments were done in order to establish some baseline data concerning the gestational age and fetal weight relation-

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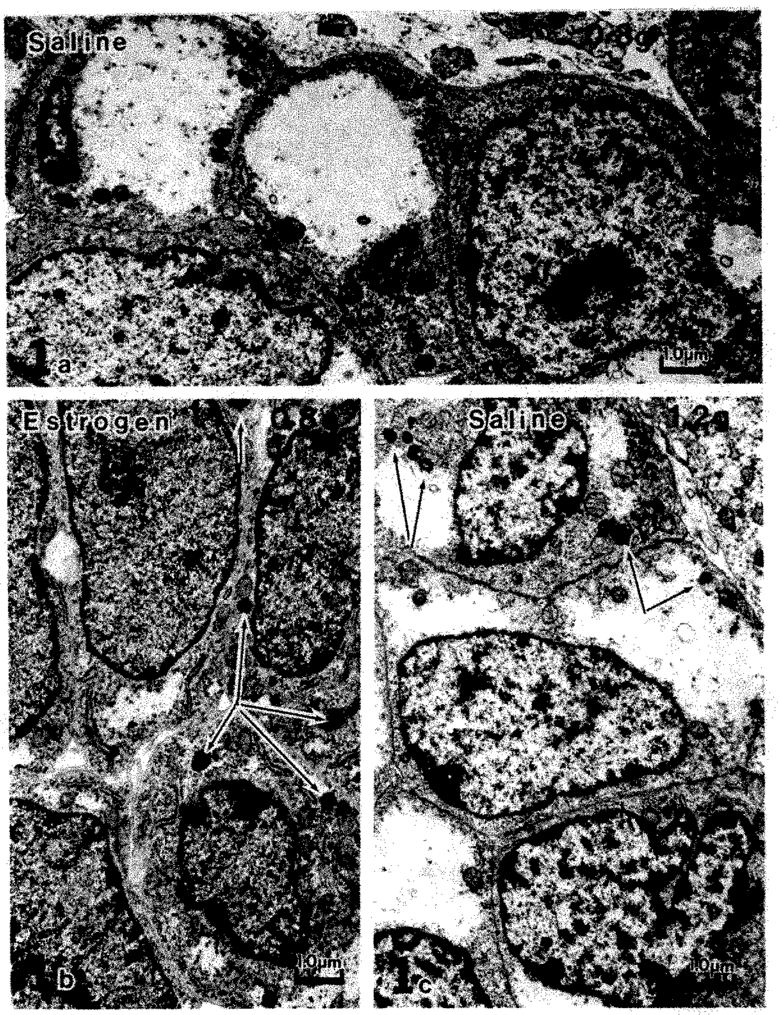

Fig. 1. Electron micrographs of fetal lung tissue from various gestational ages. The weight of the fetus is indicated on the illustration. A *bar* indicates the magnification. *a*, Portion of lung tissue from gestational day 17 of a control fetus. No lamellar bodies were present in the cells. *b*, Small lamellar bodies were present in the estrogen-exposed littermate. *c*, On gestational day 18 some small lamellar bodies could be found in a few saline solution–treated fetuses.

ship, as well as the appearance of lamellar bodies and secreted surfactant in the fetal lung.

Estrogen or saline solution injection in experimental animals. Between the fifteenth and the twentieth day of pregnancy the amniotic sacs of control and experimental fetuses of 13 animals were injected with saline solution or estradiol, respectively, and put to death 48 hours later.

Propranolol injection of experimental animals. The pregnant rats were injected subcutaneously with a dose of 3 mg/kg of body weight every 12 hours. Two animals were injected from day 17 and put to death on days 18 and 19, respectively, and one was injected from day 18 and put to death on day 20. Two rats were injected with propranolol from day 16, the amniotic sacs were injected with estrogen or saline solution on day 17, and the animals were put to death 48 hours later.

Tissue preparation for electron microscopy. The fetal lungs were rapidly removed and a thin strip close to the midline and extending from the top to the bottom was excised from the left lung. In some instances, strips were also taken from the central portion of the right lung to see if there were differences in the maturation process under controlled conditions. Ultrastructural evaluation of these did not reveal any difference in the development of the pulmonary parenchyma, and therefore the sampling was mainly restricted to the left lung strip.

Most strips were fixed by immersion but some were flooded with fixation medium immediately after the thoracic cavity had been opened. Perfusion through the trachea or pulmonary artery was not possible for practical reasons. However, since there was no air present which could prevent the penetration of fixative, a reasonable quality of fixation could still be achieved.

A highly purified 2.5% glutaraldehyde, containing

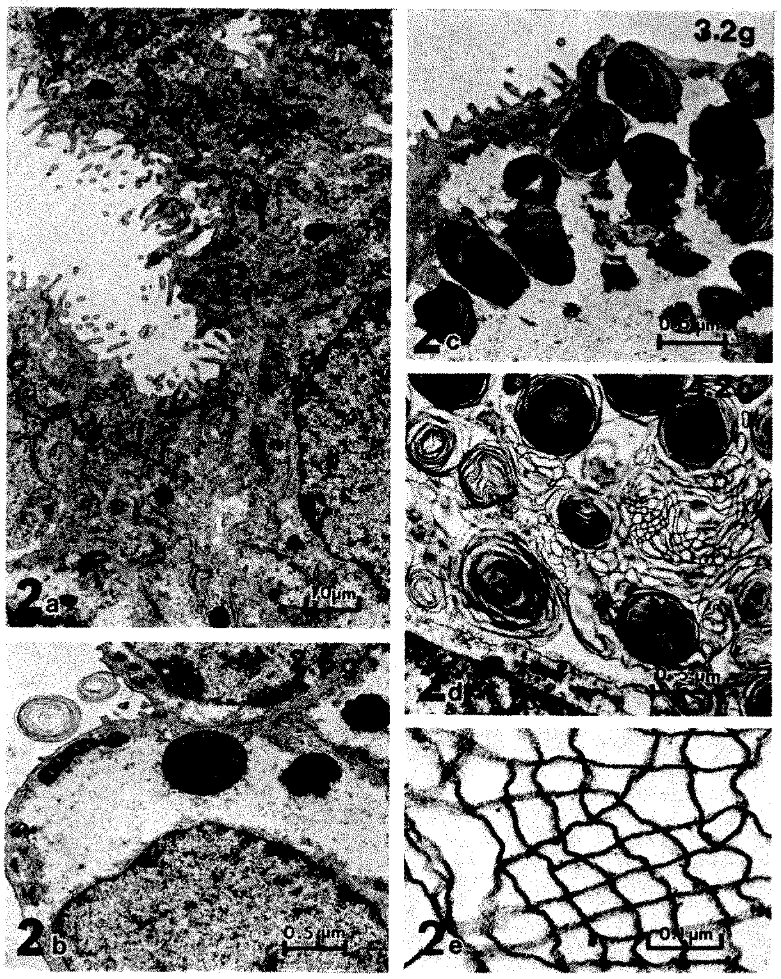

Fig. 2. Electron micrographs of fetal lung tissue from various gestational ages. The weight of the fetus is indicated on the illustration. A *bar* indicates the magnification. *a*, During gestational day 19 numerous lamellar bodies could be found in control lung tissue and microvilli were relatively well developed. *b*, On gestational day 20 multilamellar bodies were well developed and some released material could usually be found in the alveolar space. *c*, On gestational day 21 the type II alveolar cells contained numerous multilamellar bodies which were in a position to be released. *d*, Late on gestational day 21 or during day 22, immediately before spontaneous delivery, the alveolar space contained dispersing multilamellar bodies which formed the netlike surfactant structure. *e*, A portion of surfactant with typical netlike structure at higher magnification.

0.1 to 0.15 mol/L of potassium phosphate buffer (pH 7.2 to 7.4) and 0.05 mol/L of sucrose, was used as primary fixative. Postfixation was in a 2% osmium tetroxide solution in the same buffer. After rinsing, the tissue was stained en bloc in 2% cold aqueous uranyl acetate, which was gradually warmed to 40° C. This procedure was found to improve the preservation of the multilamellar bodies but also to cause extraction of glycogen. After staining, the tissue blocks were dehydrated in a graded ethanol series and embedded in Spurr's low-viscosity medium, and gray- to silver-colored sections were taken with an LKB Ultrotome. To enhance contrast the sections were stained with lead citrate on the grid before viewing under the electron microscope.

Results

The fate of the injected estrogen was determined by means of tritium-labeled estradiol phosphate. In this set of experiments the same protocol was followed, as described above, and various organs were then weighed, homogenized, and analyzed for radioactivity. At 24 hours after injection, it was found that significant amounts of radioactivity remained in the fetal body, especially the liver but also in the lungs. Very little radioactivity remained in the amniotic fluid and membranes. At 48 hours after injection the radioactivity had disappeared from the lungs and from the amniotic fluid and membranes, and only traces remained in the liver. It was also found that adjacent fetuses and amniotic

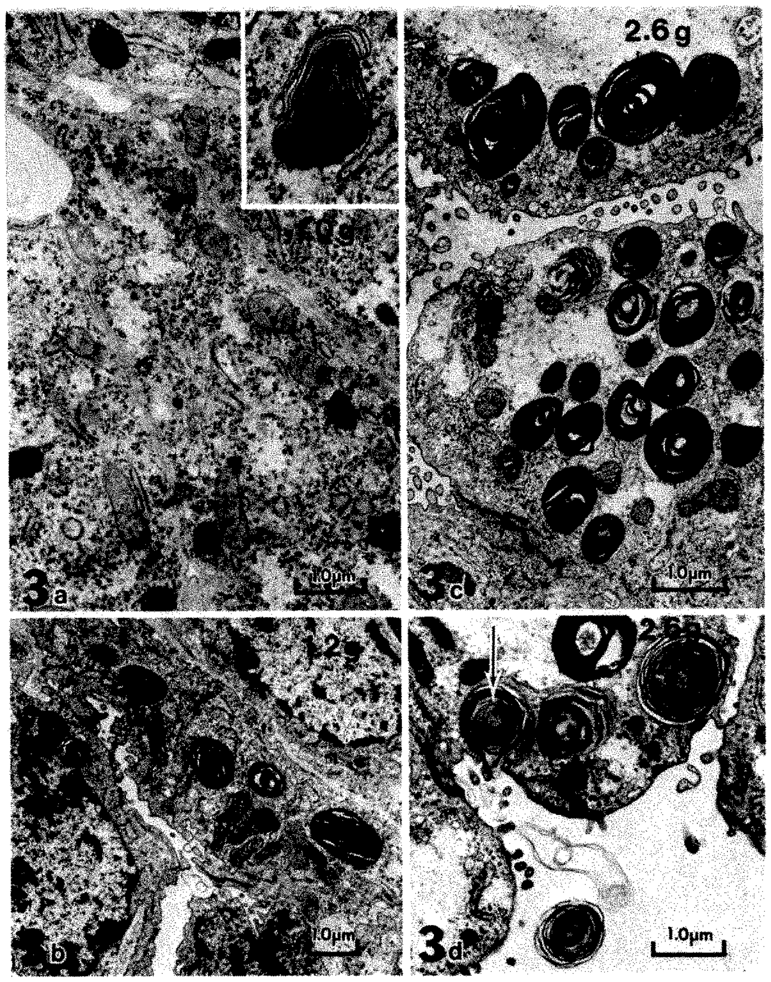

Fig. 3. Electron micrographs of fetal lung tissue from various gestational ages. The weight of the fetus is indicated on the illustration. A *bar* indicates the magnification. *a,* A portion of lung tissue from an estrogen-exposed fetus that contained numerous lamellar bodies. A saline solution–treated control littermate had not developed any lamellar bodies. Some multilamellar bodies were associated with the Golgi apparatus *(inset)*. *b,* On gestational day 18, many of the multilamellar bodies in estrogen-exposed fetuses appeared to be almost fully developed and measured 1 μm or more in size. *c,* On gestational day 20, estrogen-exposed fetuses had type II alveolar cells which were as well developed as those found on day 21 in control fetuses. *d,* The release of lamellar body contents was often observed in estrogen-exposed fetuses on gestational day 20.

sacs were not contaminated with the injected estrogen at either time. The maternal lungs, liver, and kidney had no radioactivity 24 or 48 hours after injection.

Starting on day 16 of the 22 ± 1 days of gestation the development of the lamellar bodies in the lung parenchyma was observed until immediately prior to delivery, that is, in fetuses that were already descending (Figs. 1 to 3). The formation of the multilamellar bodies, their release, and the subsequent formation of surfactant followed a progressive and sequential pattern.

On gestational day 17 (fetal weight, 0.8 to 1.0 gm) the type II alveolar cells were difficult to distinguish in noninjected and saline solution–injected fetuses (Fig. 1, *a*). No dense lamellar bodies or microvilli were present. Thus this stage probably corresponded to the late canalicular or early alveolar stage of fetal development. While the control fetuses lacked lamellar bodies, the estrogen-exposed littermate (Fig. 1, *b*) had already begun to develop bodies with a few concentric lamellae around an inner core of more homogeneous material. However, most of these lamellar bodies were only 0.25 to 0.50 μm.

Not until day 18 and in only one of the saline solution–injected fetuses with a fetal weight of 1.2 gm was the first indication of multilamellar body formation observed (Fig. 1, *c*). The fetal lung of this animal contained many cells with small dense bodies scattered in

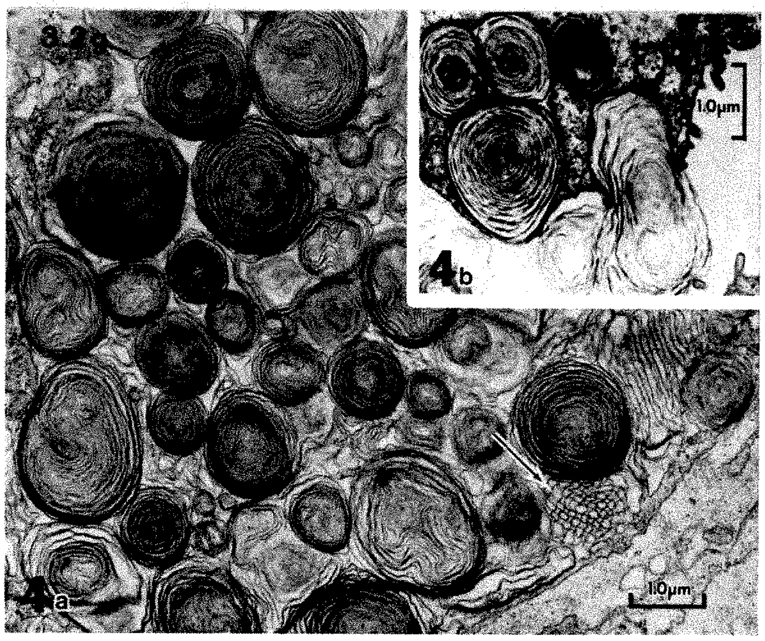

Fig. 4. Electron micrographs of fetal lung tissue from various gestational ages. The weight of the fetus is indicated on the illustration. A *bar* indicates the magnification. *a*, On gestational day 21 the extracellular space contained multilamellar bodies reaching 1 to 1.5 μm in size. Netlike surfactant *(arrow)* was often present. *b*, A releasing lamellar body and several bodies in the periphery of a type II alveolar cell, which were typical for this stage of gestation in estrogen-treated fetuses.

the cytoplasm. Eighty percent of these measured 0.25 to 0.50 μm and thus appeared to be at the same stage of development as the lamellar bodies in the 0.8 gm estrogen-exposed fetus.

The number as well as the size of the individual lamellar bodies increased from day 18 to day 19 (fetal weight, 1.2 to 1.6 gm) but 50% of the bodies were still between 0.25 and 0.50 μm in size. At day 19 it was usually possible to find microvilli (Fig. 2, *a*). In general, the process of an exocytotic release of lamellar body contents had not started during the nineteenth day of development and the occurrence of lamellar material in the alveolar space (Fig. 2, *b*) before day 20, when the fetal weight was approximately 2.0 to 2.6 gm, was rare.

Between day 20 and day 21 the fetal weight reached 3 gm. At this point in development the alveolar type II cells contained many lamellar bodies measuring 0.5 to 1.0 μm. Many of the bodies were located peripherally (Fig. 2, *c*), and several had actually fused with the cell membrane and were releasing their contents. The previously extensive number of other cell organelles was greatly reduced.

From day 21 there was an increase in secreted lamellar bodies and the characteristic netlike pattern of surfactant became evident. In the lungs of the descending fetus fixed at day 22 of gestation (weight, about 4 gm) there were large patches of material with typical surfactant ultrastructure (Fig. 2, *d*). As the lamellae were dispersed from the multilamellar body they became interwoven and coalesced to form areas with the netlike substructure (Fig. 2, *e*).

Estrogen-exposed fetuses. Multilamellar bodies could be observed from the seventeenth day of gestation when the fetus weighed ≥0.8 gm and had been exposed to estrogen since day 15 (Fig. 1, *b*). If the amniotic sac was injected prior to day 15 the fetuses did not survive. Also, in surviving fetuses weighing < 0.8 gm it was not possible to find lamellar bodies.

On day 17 many small lamellar bodies were scattered in the cytoplasm of presumptive alveolar type II cells. They were similar to those observed in fetuses 1 to 2 days older, which had not been subjected to estrogen exposure (compare Fig. 1, *b*, with 1, *c*, and 2, *a*). Late on day 17, when the fetal weight was 1 gm (Fig. 3, *a*), about 75% of the lamellar bodies were larger than 0.5 μm. At this time many cells were undergoing mitosis and an abundance of ribosome-studded endoplasmic reticulum, free ribosomes, and well-developed Golgi apparatus were present. The Golgi cisternae were often in close contact with developing multilamellar bodies (Fig. 3, *a*, *inset*), indicating that some of the material was packed via the Golgi apparatus.

On day 18, when the fetal weight was 1.2 gm (Fig. 3, *b*), the multilamellar bodies were more developed than in saline solution–injected fetuses weighing 1.6 gm (Fig. 2, *a*). Many bodies measured ≥1.0 μm and were thus usually larger than the mitochondria. Only

Table I. The number and size of lamellar bodies in estrogen-treated rat fetuses versus saline solution–injected littermates of same weight at various stages of gestation

	Control fetuses (No. of bodies)						*Estrogen-treated fetuses (No. of bodies)*				
Fetal age (days)	17-18		18-19		19-21		17-18		18-19	19-20	21
Fetal weight (gm)	0.8	1.0	1.2	1.6	1.6	3.2	0.8	1.0	1.2	1.2	3.2
Size of bodies (μm)											
0.25-0.50	—	—	80	60	20	25	89	27	20	10	14
0.50-0.75	—	—	16	45	49	46	18	79	25	40	25
0.75-1.00	—	—	4	15	67	80	3	34	70	62	51
1.00-1.25	—	—	—	1	9	44	—	—	55	83	47
1.25-1.50	—	—	—	—	4	20	—	—	40	46	83
1.50-1.75	—	—	—	—	—	3	—	—	—	13	64
>1.75	—	—	—	—	—	—	—	—	—	2	20
Total No.	—	—	100	121	149	218	110	140	210	256	304

10% of the lamellar bodies were <0.5 μm in diameter, compared to 80% in the 1.2 gm control animal.

On day 19 to day 20 many lamellar bodies appeared to be fully developed in structure and size (Fig. 3, *c*). Release of lamellar bodies was observed in estrogen-treated fetuses weighing <2 gm and became abundant during day 20 (Fig. 3, *d*).

By early on day 21, when the fetus weighed 3.2 gm, the entire alveolar cavity was sometimes filled with released lamellar bodies and material with the distinct structure of surfactant (Fig. 4, *a*). Numerous lamellar bodies still within the alveolar cells were close to the cell surface or undergoing exocytosis (Fig. 4, *b*). Thus the maturity of the type II alveolar cells and the lamellar bodies could be favorably compared to the stage reached in control fetuses that were already descending.

The difference in the number and the size of the lamellar bodies in the type II alveolar cells of estrogen-treated fetuses and littermate controls (injected with saline solution) of the same weight is shown in Table I. In each animal, 20 alveolar cells were selected at random and carefully screened for lamellar bodies. At fetal weights of 0.8 and 1.0 gm, corresponding to early and late on day 17, a good many, albeit small, lamellar bodies were found in the alveolar cells of estrogen-treated animals, but none in the controls. From day 18 there was a successive increase in the number and mean size of lamellar bodies in both the treated and the control animals. However, in both respects saline solution–injected animals lagged approximately 2 days behind those that had received estrogen (Fig. 6, *A* and *B*).

Estrogen-propranolol interactions. Only a few lamellar bodies were present in the fetal lungs at a gestational age of 18 or 19 days when the mother had been injected with propranolol from day 17. Also, with injection from day 18 and sacrifice on day 20 the number of lamellar bodies was low and many of those present were abnormal (Fig. 5, *a*). Large lipid droplets were scattered in the cytoplasm, but there were fewer ribosomes and the Golgi apparatus was not well developed (Fig. 5, *b*). When the mother was injected with propranolol on day 16 and the fetuses exposed to estrogen on day 17 and put to death 48 hours later, the number of lamellar bodies remained low and there was no significant difference compared to the number in saline solution–injected or noninjected littermates (Fig. 5, *c*).

Comment

The results of these experiments clearly indicate that estrogen injected into the fetal compartment in pregnant rats enhances the formation and subsequent release of lamellar bodies from type II alveolar cells in the fetal lungs. This appears to be accomplished through the increased synthesis and packaging of the surface-active phospholipids in the lamellar bodies, as indicated by the large number of ribosomes and the apparent increase in quantity of the rough endoplasmic reticulum. It is also clear that these changes cannot be attributed to stress induced by manipulation and trauma of the operative procedure or by invasion of the amniotic sac, since the saline solution–injected fetuses did not show the response that was observed after estrogen treatment.

There is considerable circumstantial evidence that adrenergic mechanisms, or at least β-adrenergic receptors, are involved in the process of surfactant release from type II alveolar cells. It has been documented that β-adrenergic–receptor agonists greatly enhance the release of disaturated phosphatidylcholine, the major phospholipid component in surfactant, from murine type II cells in culture.[9] Oyarzun and Clements[12, 13] found that systemic administration of terbutaline in rabbits caused an increase in the phospholipid content in lung lavages and that this increase, as well as that induced by hyperventilation, was blocked by propranolol. Also in the rat, injection of a β-adrenergic–receptor agonist has been reported to increase the phospho-

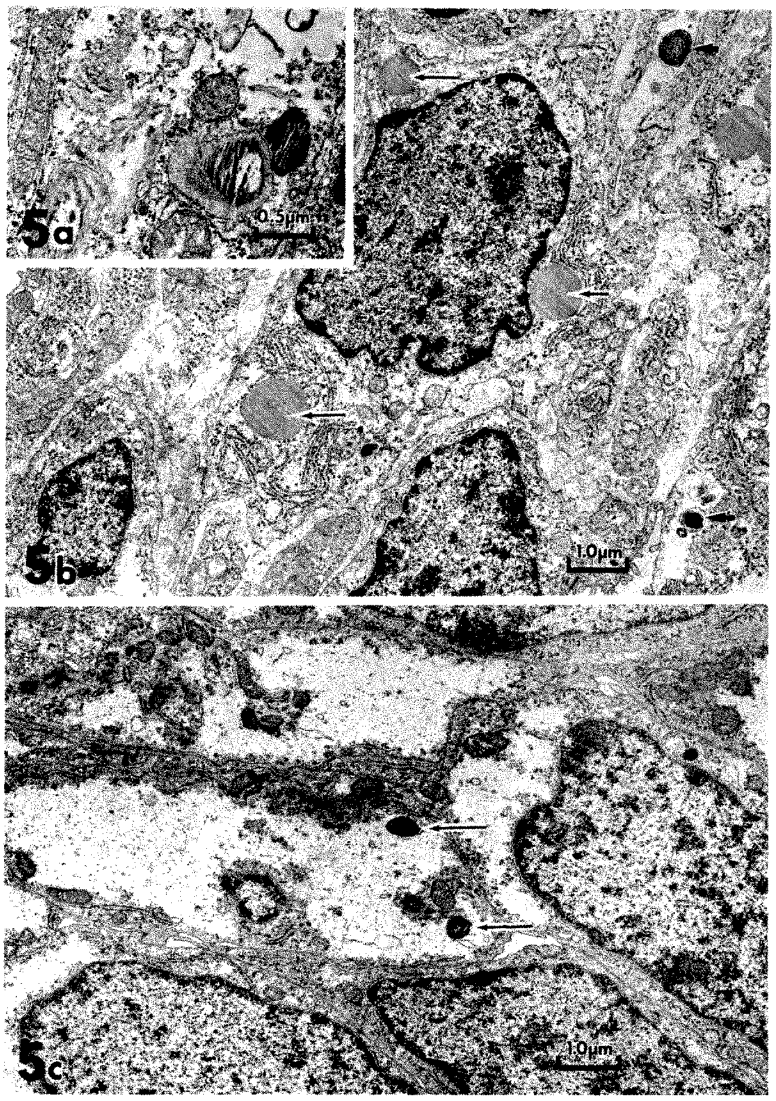

Fig. 5. Electron micrographs of fetal lung tissue from various gestational ages. A *bar* indicates the magnification. *a*, After propranolol exposure, on gestational day 20 the fetuses had a diminished number of lamellar bodies and those present were often distorted. *b*, On gestational day 20, large lipid droplets *(arrows)* were present in many of the type II alveolar cells with only a few lamellar bodies *(arrowheads)* after propranolol treatment. *c*, Estrogen injection 24 hours after the initial propranolol exposure did not significantly reverse the inhibitory effect of propranolol. Only a few lamellar bodies were present on day 19 of gestation.

lipid content in lung lavages.[14] In this case, however, there was a concomitant decrease in the number of lamellar bodies per type II "cell profile." The interpretation of these results was that the functional effect of β-adrenergic stimulation is release of preformed lamellar bodies rather than de novo synthesis.

Recently it was shown that estrogen increases the number of β-adrenergic–binding sites in the lungs of mature and immature rabbits[10] as well as rabbit fetuses.[15] It is reasonable to assume, therefore, that the increased release of lamellar bodies after treatment of rat fetuses with estrogen observed in the present study was in fact due to activation of the β-adrenergic mechanism. However, in contrast to treatment with β-adrenergic–receptor agonists, there was also a marked increase in the formation of new lamellar bodies in the type II alveolar cells. At present it is not clear whether this is also the result of the activation of β-adrenergic receptors or whether it represents a separate mechanism, possibly the enhancement of protein synthesis, which is suggested by the abundance of endoplasmic reticulum in the type II cells of estrogen-treated fetuses.

We also observed that administration of propranolol to the mother greatly retarded both the formation and the release of lamellar bodies in the fetal lung. More-

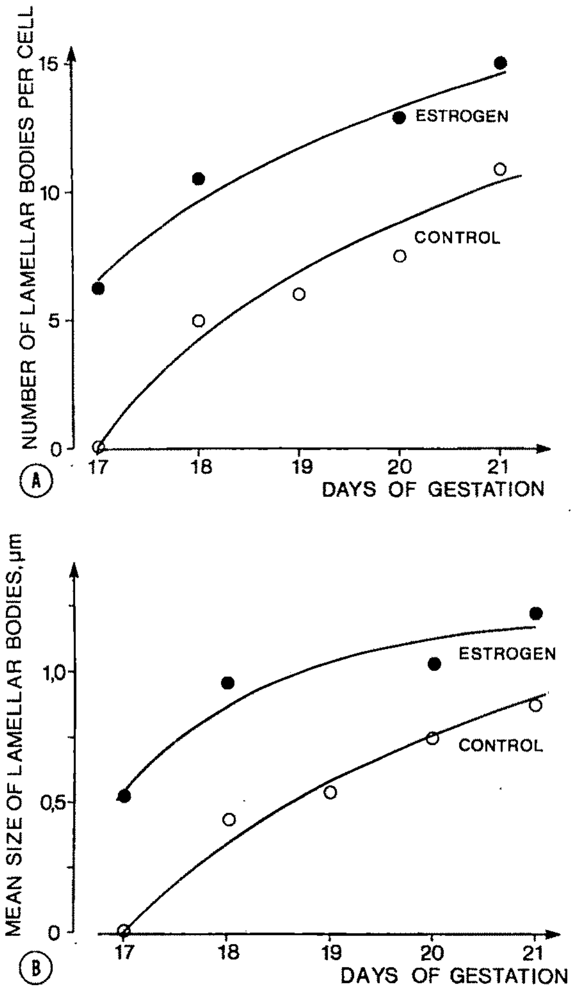

Fig. 6. The number of lamellar bodies *(A)* and their mean size *(B)* in the type II alveolar cells of rat fetuses at various stages of gestation. The animals were littermates of equal weight and had been injected with estrogen or saline solution (control) 48 hours before sacrifice. Each *symbol* represents the mean value from 20 cells chosen at random.

over, exposure of the fetus to estrogen did not reverse this effect. This seems to indicate that the β-adrenergic receptors may control the entire chain of events leading ultimately to surfactant being presented to the alveolar wall and that estrogen is capable of stimulating lamellar body synthesis and release only when a sufficient number of β-adrenergic receptors are available. However, these data should be interpreted cautiously because propranolol was given either before or when only a few lamellar bodies had appeared in the fetal lungs. Moreover, the dose used was high and apparently caused some trauma to the fetuses, as indicated by the slightly reduced weight in these animals and the presence of abnormal lamellar bodies in some of the type II cells. Further experiments with the use of β-adrenergic–receptor antagonists in lower doses and at various stages of gestation are required to elucidate the presumed interplay between estrogen and the β-adrenergic–receptor mechanism in the synthesis and/or release of lamellar bodies.

Regardless of its mechanism of action, estrogen must be added to the list of endogenous agents that stimulate surfactant formation in and release from the type II cells of the fetal lung. It is of interest to note that the plasma levels of estrogen increase near term and those of progesterone decrease, a change apparently brought about by a surge of cortisol secreted from the fetal adrenal gland and affecting steroid production in the placenta.[16] Both cortisol and estrogen raise pulmonary β-adrenergic–receptor activity[10, 17] and promote fetal lung maturation,[18] whereas progesterone has an inhibitory effect, at least on the β-receptors.[10]

At present, the significance of estrogen in the process of lung maturation is not known, but it is striking that the change in the estrogen/progesterone ratio coincides with development and maturation of lamellar bodies in the fetal lung and that premature delivery, before the rise in estrogen, is associated with an increased incidence of respiratory distress syndrome.

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Estrogen increases β-adrenergic binding in the preterm fetal rabbit lung

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Tritium-labeled dihydroalprenolol was used to quantify β-adrenergic–receptor sites in day 28 fetal rabbit lung tissue. Each of the amniotic sacs of pregnant New Zealand White rabbits on day 26 of gestation was injected in vivo with estrogen (estradiol phosphate, 1.6 μg) in one horn and normal saline solution in the contralateral one. The animals were put to death 48 hours later and the fetal lung tissues were assayed. Estrogen increased the number of β-adrenergic–receptor sites in the treatment group compared to the control group (216 versus 163 fmol/mg of protein, $p < 0.02$ by paired t test). In the presence of estrogen, β-adrenergic–receptor activity is enhanced in the preterm fetal rabbit. This effect may be implicated in the β-adrenergic mediation of phospholipid synthesis and release in fetal alveolar cells. (Am J Obstet Gynecol 1985;151:514-9.)

Key words: β-Adrenergic receptor, estrogen, fetal rabbit lung, phospholipid synthesis and release, surfactant

Our aim in this study was to assess the effect of estrogen on the lung β-adrenergic–receptor sites in rabbit fetuses in vivo, as determined by radioligand binding assays. Previously we have reported that exogenous estrogen and endogenous estrogen increase the number of β-adrenergic–receptor sites in immature and mature rabbit lung tissue.[1] β-Adrenergic stimulation has been shown to accelerate surfactant production in the fetal lung.[2,3] Given that estrogen affects the number of β-adrenergic–receptor sites in mature and immature rabbit lungs, these experiments were designed to investigate whether the same mechanism is functional during fetal development. It is also important to know whether this mechanism is implicated in the process of synthesis and release of the lung surfactant.

Material and methods

We used tritium-labeled dihydroalprenolol, a reversible and potent β-adrenergic agonist with pharmacologic characteristics consistent with binding to β-adrenergic receptors, to quantify the numbers of β-adrenergic–receptor sites in the fetal New Zealand White rabbit lung following estrogen treatment.

Pharmacologic agents. Dihydroalprenolol with a specific activity of 49.1 Ci/mmol was obtained from New England Nuclear. (−)-Norepinephrine hydrochloride, (−)-epinephrine bitartrate, (−)-alprenolol, (−)-isoproterenol hydrochloride, (+)-isoproterenol hydrochloride, and estradiol phosphate were obtained from Sigma Chemical Company. (+)-Propranolol, (−)-propranolol, and (+)-norepinephrine bitartrate were a gift from Dr. Leon Goldberg, Department of Pharmacology, The University of Chicago.

Treatment regimen. Six mature female New Zealand White rabbits were bred, with day zero of gestation taken as the day of breeding. On day 26 of gestation the rabbits were anesthetized with 40 mg/kg of ketamine, 16 mg/kg of xylazine, and 2.5% halothane gas. The two uterine horns were exposed and 1.6 μg of estradiol phosphate in 20 μl of sterile saline solution was injected via a 30-gauge needle into the amniotic sacs of each of the fetuses on one side. The fetuses in the contralateral uterine horn received 20 μl of sterile saline solution alone. The maternal abdomen was closed and the rabbit allowed to recover. Each rabbit

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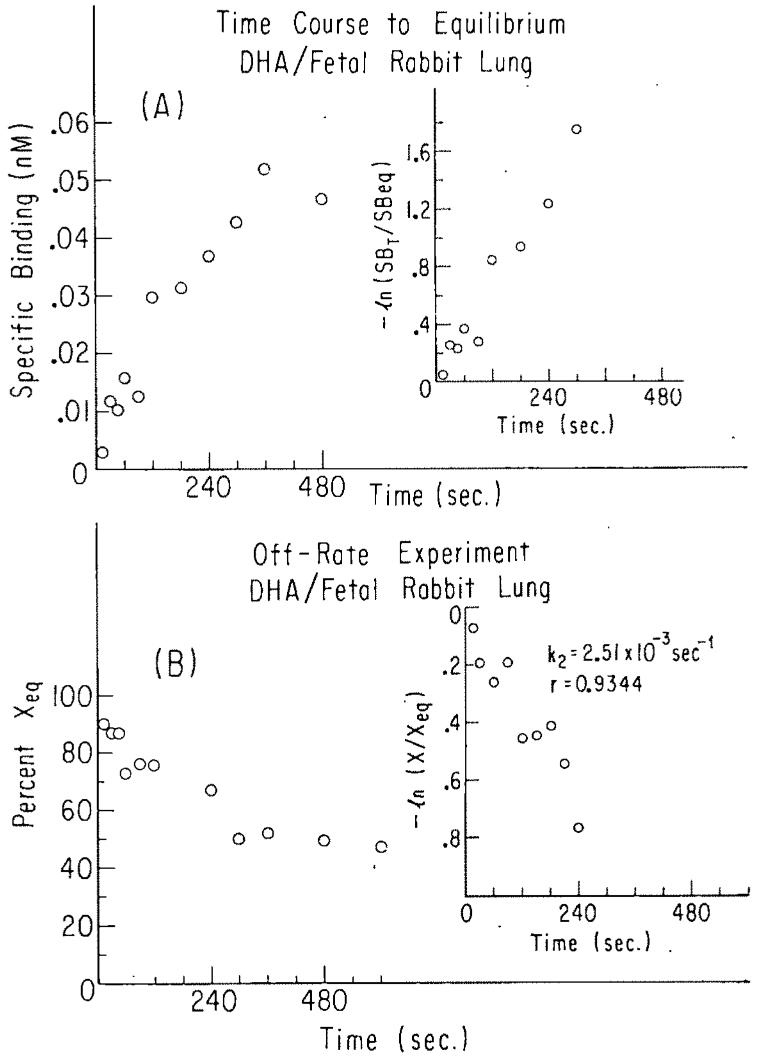

Fig. 1. *A,* Time course to equilibrium for specific dihydroalprenolol binding. The inset shows the pseudo–first-order kinetic plot of dihydronalprenolol binding. *SB_t* represents the concentration of specific bound dihydroalprenolol at time t; *SB_{eq}*, the concentration of dihydroalprenolol specifically bound at equilibrium. The slope of the line defined by the equation $\ln[SB_{eq}/(SB_{eq} - SB_t)] = K_{obs}$ is 7.9×10^{-3} sec^{-1}. From the relationship $K_1 = (K_{obs} - K_2)/[DHA]$, where K_2 is the first-order rate constant for reversal (2.652×10^{-3} sec^{-1}; see Fig. 1, *B*), $K_1 = 5.1361 \times 10^{-4}$ nmol/L^{-1} sec^{-1}. Parallel experiments (not shown) demonstrated that the dihydroalprenolol-binding site complex was stable for more than 1 hour. *B,* The reversal of dihydroalprenolol binding. After dihydroalprenolol binding reached equilibrium, nonlabeled alprenolol was added to a concentration of 10 μmol/L (time zero). The *inset* shows the first-order reversal of the dihydroalprenolol-binding site complex; the line is determined by the equation $-\ln(SB_t/SB_{eq}) = K_2t$. From these data, $K_2 = 2.65 \times 10^{-3}$ sec^{-1} and t½ = 377 seconds.

was put to death on day 28 of gestation and the fetuses delivered by hysterotomy. A total of 43 fetuses were used; for each litter, the lungs of the fetuses receiving estrogen were pooled, as were those of the control fetuses. There were two stillbirths in this study series and none of the animals delivered fetuses prematurely.

Preparation of lung tissue. The fetal lungs were removed intact, weighed, and placed immediately into ice-cold Tris buffer (50 mmol/L, pH 7.4). The lung tissue was homogenized for three 15-second intervals at 0° C on a Brinkman Polytron homogenizer at one half speed. Thirty-second cooling periods separated each homogenization.

The homogenate was centrifuged at 315 × g for 12 minutes in a Beckman RC-58 refrigerated centrifuge at 0° C to remove cellular debris. The supernatant was centrifuged at 11,400 × g for 15 minutes, and the membrane pellet was then resuspended in 1 ml of Tris buffer, 50 mmol/L (pH 7.4). The specimens were flash frozen and stored in liquid nitrogen and assayed within 1 week. Protein concentration was determined by the

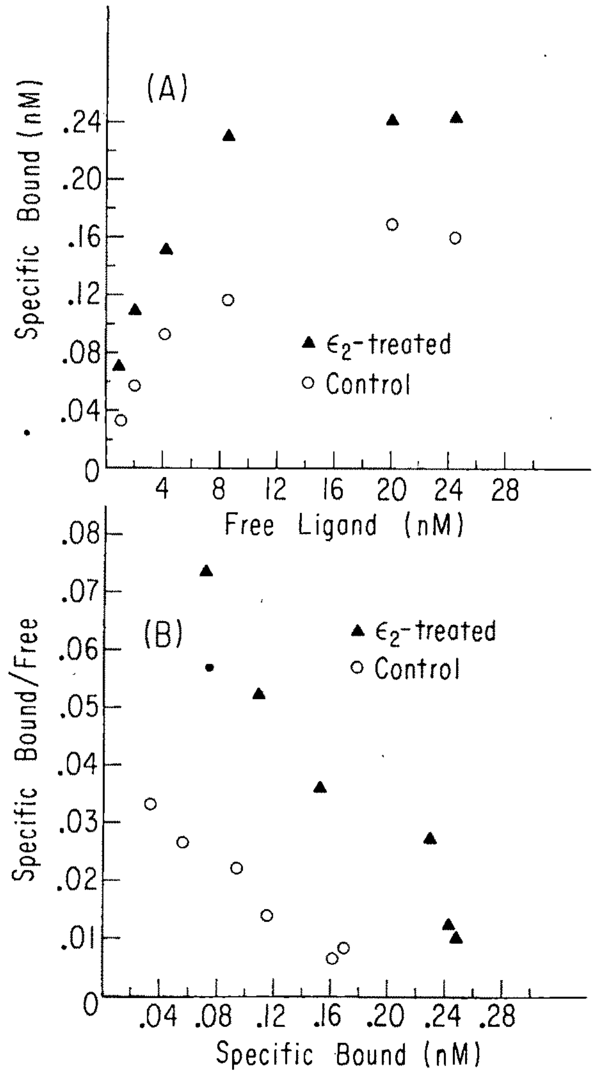

Fig. 2. *A*, Direct plot of specific dihydroalprenolol binding. *B*, Scatchard analysis of dihydroalprenolol binding to rabbit lung membrane fraction (1.0 mg/ml of protein concentration).

method of Bradford,[4] with bovine serum albumin used as the standard.

Dihydroalprenolol binding assay. Dihydroalprenolol was diluted in Tris buffer, 50 mmol/L (pH 7.4). Nonlabeled alprenolol at a concentration of 1000 × the dissociation constant (K_D) was used to define nonspecific binding. The assay and all binding were performed in a total volume of 0.25 ml, consisting of 1.25 to 25 nmol of dihydroalprenolol, 0.25 mg of membrane protein, and 0.1 mmol/L of hydrochloric acid by itself or with added adrenergic agents. Incubation was for 15 minutes at 30° C and was terminated by the addition of 5 ml of ice-cold Tris buffer, 50 mmol/L. The samples were then immediately filtered over Whatman GF/C filters under vacuum. The tubes were rinsed with 5 ml of wash buffer, and the filters were then washed with an additional 5 ml of wash buffer. The filters were dried under increased vacuum and then counted in Biofluor (New England Nuclear) scintillation fluid in a Beckman LS-8000 automatic scintillation counter. Specific binding was determined by subtracting radioactivity bound in the presence of 10 µmol/L of alprenolol, a concentration of approximately 1000 × the K_D of dihydroalprenolol in this tissue, from that bound in the presence of dihydroalprenolol alone. Specific binding constituted 50% to 60% of total radioactivity bound and was a linear function of added membrane protein across the concentration range used in this study. K_D and the number of dihydroalprenolol-binding sites were calculated by means of Scatchard plotting and linear regression analysis.

Results

Dihydroalprenolol binding to fetal rabbit lung membrane fraction. A series of experiments were performed in order to verify that dihydroalprenolol binding was consistent with β-adrenergic–receptor activity. Dihydroalprenolol binding should be rapid, reversible, and saturable, and adrenergic competitors should show a rank order of potencies parallel to their pharmacologic potencies and compete for binding stereoselectively, with (−)isomers much more potent than (+)isomers.

The kinetics of dihydroalprenolol binding are illustrated in Fig. 1, *A* and *B*. Equilibrium was reached by 8 minutes and remained stable in excess of 1 hour. The addition of 10 µmol/L of alprenolol (Fig. 1, *B*) demonstrates the reversibility of dihydroalprenolol binding, with a half-time (t½) of approximately 240 seconds. The dissociation of specifically bound dihydroalprenolol followed first-order kinetics, with a rate constant of reversal (K_2) of 2.5120 × 10^{-3} sec^{-1}. The forward rate constant (K_1), determined from the relationship $K_1 = (K_{obs} - K_2/[DHA])$, where K_{obs} = observed rate constant and DHA = dihydroalprenolol, was 5.1361 × 10^{-4} $nmol/L^{-1}$ sec^{-1}, yielding a kinetically derived estimate of the K_D for dihydroalprenolol binding ($K_D = K_2/K_1$) of 4.89 nmol/L.

Equilibrium studies across a range of dihydroalprenolol concentrations show that specific binding is saturable (Fig. 2, *A*), with 163 ± 12 fmol (mean ± SEM) bound per milligram of protein in lung tissue from fetal control rabbits. Scatchard analysis (Fig. 2, *B*) indicated that this binding is to a single class of receptors. The mean value of K_D from these experiments was 4.35 ± 0.82 nmol/L, in good agreement with the kinetically derived value.

The results of competitive binding experiments are shown in Fig. 3, *A* to *D*. For isoproterenol, propranolol, and norepinephrine (Fig. 3, *A* to *C*), the (−)stereoisomers were far more potent than the (+)stereoisomers. The biologically inactive (+)isomer of norepinephrine

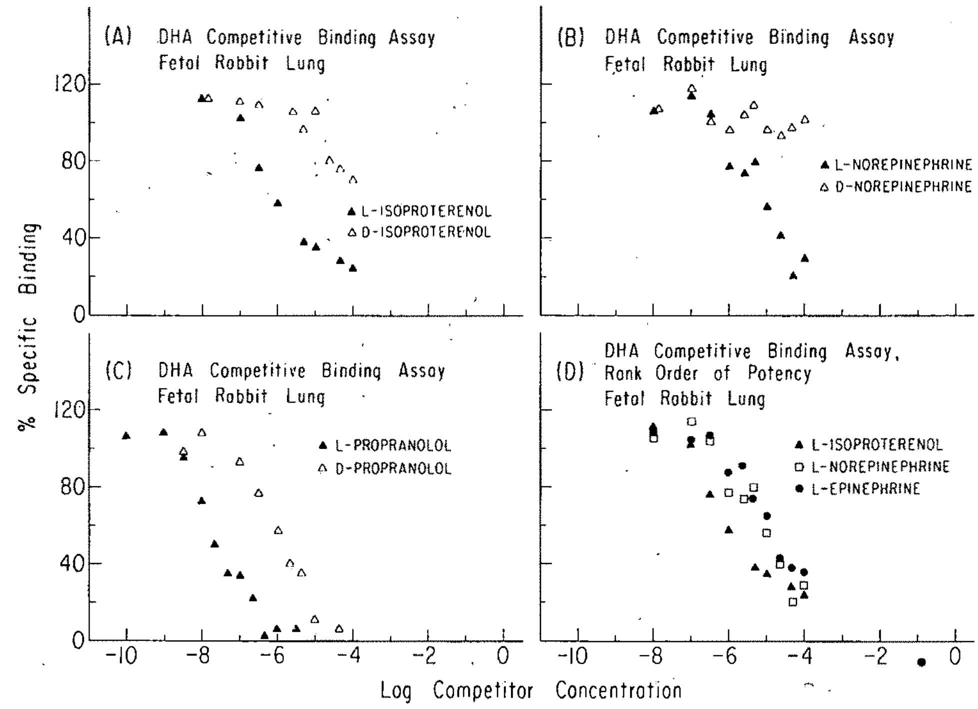

Fig. 3. *A* to *C*, Competitive binding assays showing the comparison between the (+)stereoisomers and (−)stereoisomers of isoproterenol, propranolol, and norepinephrine. The (+)isomers of isoproterenol and norepinephrine failed to compete at the highest concentration tested (100 μmol/L. *D*, Rank order of potency for the (−)isomers of isoproterenol, norepinephrine, and epinephrine. The concentration of dihydroalprenolol was 9 nmol/L.

failed to compete with dihydroalprenolol at the highest concentration tested (0.1 mmol/L). Fig. 3, *D*, shows that the relative rank order of competitive potencies was (−)-isoproterenol >> (−)-norepinephrine = (−)-epinephrine. This sequence parallels the order of potency of the pharmacologic effect of these three drugs on the β_1-subpopulation of adrenergic receptors.

Effect of estrogen on dihydroalprenolol binding. As shown in Fig. 4, estrogen treatment led to a significant elevation of β-adrenergic–receptor sites in preterm fetal rabbits compared to those seen in untreated controls (216 ± 24 versus 163 ± 12 fmol/mg protein, mean ± SEM, $p < 0.02$ by paired *t* test). A paired *t* test was performed with the use of the pooled values of β-adrenergic–receptor site numbers for the estrogen-treated and control group of each of the six litters.

Comment

The present experiments demonstrate an increase in the number of fetal rabbit lung β-adrenergic–receptor sites under the influence of estrogen when compared to littermate controls. This result is consistent with our previous findings in the lung tissue of rabbits, after parturition.[1] Given the known rise in endogenous estrogen in the pregnant rabbit near term[5] and the well-documented impact of β-adrenergic–receptor activity on fetal lung surfactant production,[2, 3] it appears likely

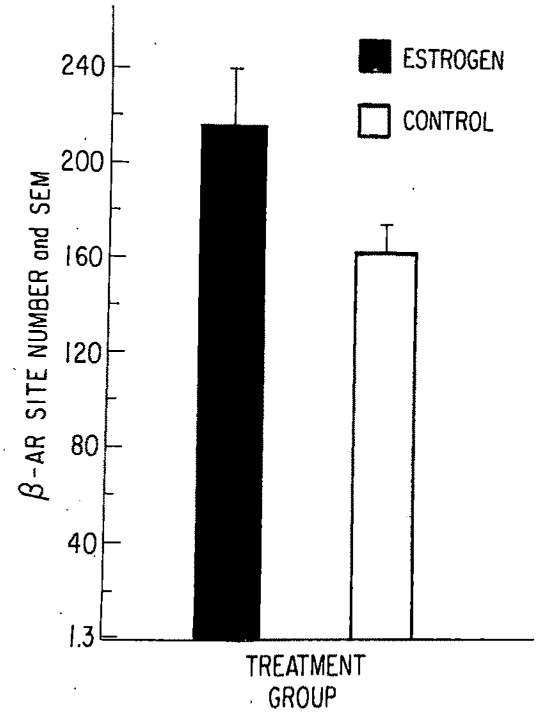

Fig. 4. Results of the dihydroalprenolol-binding assay in the two treatment groups of preterm fetal rabbits. Comparisons of the differences in the mean values of β-adrenergic–receptor site numbers between the control and estrogen group were statistically significant ($p < 0.02$ by paired *t* test).

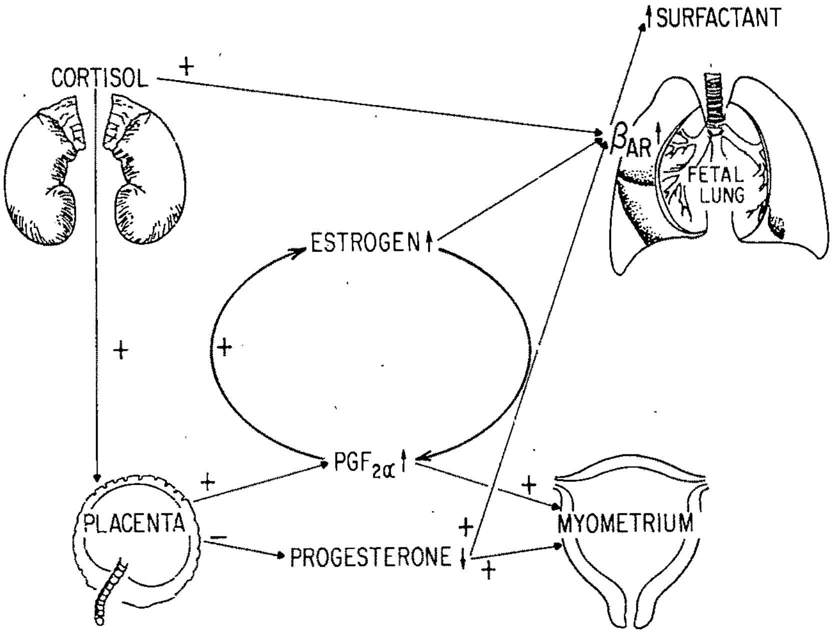

Fig. 5. Diagram, expanded from Liggins,[12] showing possible connections between fetal cortisol surge, subsequent increased estrogen and decreased progesterone, and both fetal lung maturation and preparation of the myometrium for labor.

that estrogen plays a role in the maturation process of the prenatal rabbit lung.

Because we have not studied the absorption route of estrogen from the amniotic fluid, we do not know whether it reached the fetus by swallowing, by respiratory movements, or through the fetal skin. However, since we have proper controls with saline solution injections in littermates the route of absorption is immaterial to the significance of our results—we know that the estrogen injected in the fetal compartments was the cause of the β-adrenergic lung maturation. Because the assay for β-adrenergic receptors is performed on whole-lung homogenates the question whether this binding represents receptors in the lung parenchyma, in the tracheobronchial elements, and/or in the lung vasculature must be considered. Concurrent with these experiments we have evaluated the effect of estrogen on type II alveolar cell ultrastructure and lamellar body formation in the fetal rat by means of the electron microscope.[6] These studies clearly demonstrate that estrogen exerts a positive effect on the time of the first appearance of lamellar bodies, their rate of proliferation, and their release into the alveolar space. Other investigators have found similar results with the use of light microscopy in the fetal rabbit.[7] Since direct β-adrenergic stimulation is known to produce a similar acceleration of surfactant production, this argues in favor of a direct role for estrogen in the lung parenchyma. However, other drugs and hormones, including the corticosteroids and aminophylline, stimulate β-adrenergic–receptor formation in the fetal lung.[9-11] Further work with the use of isolated type II alveolar cells in cell culture is needed to resolve these twin issues of which cell type is being stimulated and the chain of events leading to its stimulation. The model proposed in Fig. 5, while not specific to the rabbit, is useful for depicting the effects of estrogen, progesterone, and the corticosteroids on both the pregnant uterus and the fetal lung, effects which may link the onset of labor contractility to the maturation of fetal lung surfactant.

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Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor

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The production of prostaglandins by dispersed cells from human amnion, chorion, and decidua was examined at term before the onset of labor and at spontaneous vaginal delivery. In order to obtain detailed information about relative prostaglandin production rates, the time course of prostaglandin output was examined by incubating the cells for up to 4 hours and measuring the cumulative output of prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in the incubation media. The output of all three prostaglandins was low in tissues obtained before the onset of labor. At labor there was an increased production of prostaglandins E_2 and $F_{2\alpha}$ in amnion and a small increase in the output of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in decidua. In contrast, chorionic cells obtained at spontaneous vaginal delivery showed high levels of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in the media with no net production of prostaglandin E_2 or $F_{2\alpha}$. These data suggest a high rate of specific in vitro prostaglandin synthesis in amnion and decidua at labor, accompanied by a high rate of prostaglandin metabolism in chorion. (Am J Obstet Gynecol 1985;151:519-23.)

Key words: Prostaglandin, amnion, chorion, decidua

Several lines of evidence suggest a role for prostaglandins in human parturition. Exogenous prostaglandins have been shown to induce labor,[1] and in monkeys the onset of parturition can be delayed by prostaglandin synthesis inhibitors.[2] Concentrations of prostaglandins in peripheral plasma,[3] amniotic fluid,[4-6] and tissues[7] increase at labor.

The likely sources of these increased prostaglandins at parturition are the intrauterine tissues. Many researchers have reported that homogenates[8,9] and whole cells[10,11] from these tissues have the capacity to produce prostaglandins. This production has been reported to increase at labor by some authors,[11,12] while other authors[10,13] have found little or no increase. There are also conflicting reports about the relative amounts of the primary prostaglandins produced by these tissues.[10-12,14]

Some of this conflict may be due to differences in the lengths of the incubation times used in these studies. Therefore we have performed detailed time-course experiments in order to examine the effects of labor on the type of prostaglandins produced by dispersed cells from human amnion, chorion, decidua, and placenta. Prostaglandin synthesis was studied at term before the onset of labor and at spontaneous delivery. The relative rates of production of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ from endogenous precursors were examined to determine the pattern of prostaglandin production for each tissue and to measure changes in the output of individual prostaglandins with time of incubation.

Material and methods

Subjects. Fetal membranes, with attached decidua and placenta, were obtained from women who were

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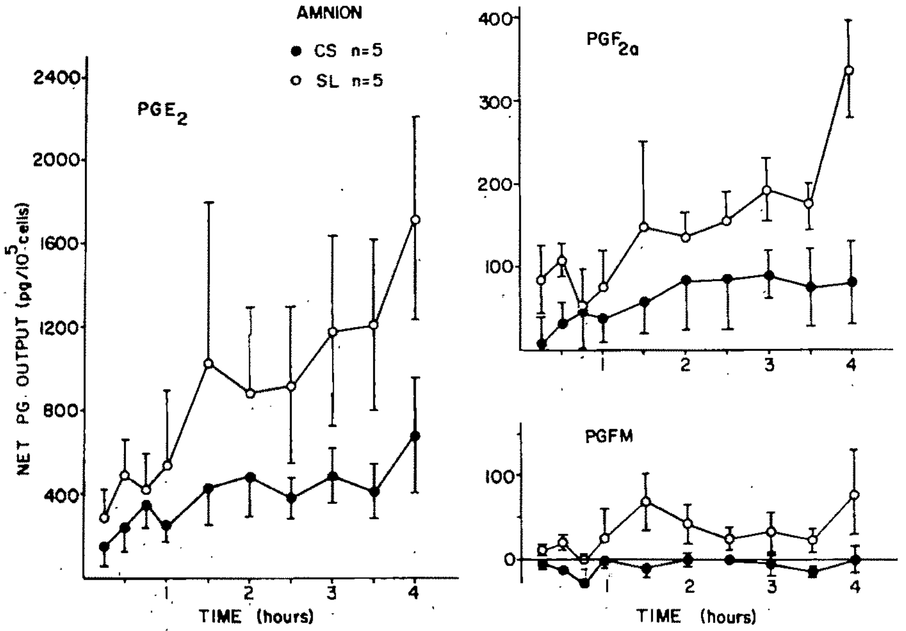

Fig. 1. The net production (mean ± SEM) of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ *(PGFM)* by dispersed cells of amnion obtained at cesarean section *(CS)* or at spontaneous vaginal delivery *(SL)*.

delivered spontaneously at term (n = 5) or from women undergoing elective cesarean section (n = 5) at term prior to the onset of labor.

Tissues. Tissues were obtained immediately after delivery. The amnion was separated manually from the chorion, and the chorion and decidua were then gently separated with fine forceps. Each tissue was rinsed several times in Dulbecco's phosphate-buffered saline (pH 7.4) to remove clotted blood.

Cell dispersal. Cells were prepared by a modification of the collagenase method of Olson et al.[11] The rinsed tissues were cut into pieces (2 cm^2 for fetal membranes and decidua) and placed in separate 250 ml Erlenmeyer flasks. Freshly prepared collagenase (0.1%, Type I, Sigma Chemical Co.) and trypsin inhibitor (0.05%, Type I-S, Sigma Chemical Co.) in Dulbecco's phosphate-buffered saline (30 ml) were added to each flask, and the flasks were tightly stoppered. The tissues were agitated for 15 to 20 minutes at 37° C in a shaking water bath. The tissue pieces were then removed and reincubated in fresh collagenase/trypsin inhibitor solution for 1 to 2 hours, with vigorous shaking every 20 to 25 minutes. The initial collagenase solution was discarded.

When the cell suspensions appeared uniform, with no pieces of tissue remaining, the cells were collected by centrifugation (125 × g, 10 minutes). The cells were then washed with phosphate-buffered saline, centrifuged (125 × g, 10 minutes), and resuspended in 15 ml of Eagle's minimum essential medium (pH 7.4, Eagle's minimum essential medium with glutamine, without sodium bicarbonate, Flow Laboratories). Cell viability was assessed using trypan blue exclusion staining, and the cells were counted with the aid of a hemacytometer. The cell suspensions were then diluted so that the concentration of viable cells was 2 × 10^5 per milliliter of minimum essential medium.

Cell incubation. The cell suspensions (0.5 ml) were added to polystyrene culture tubes (12 by 75 mm) containing 0.5 ml of minimum essential medium, giving a final cell concentration of 1 × 10^5 per milliliter. No exogenous substrates or cofactors were added. Cells were agitated gently in a shaking water bath at 37° C while incubating for various lengths of time under air. Incubations were terminated by freezing at −70° C. All incubations were performed in duplicate. Both blanks (minimum essential medium without cells) and cells in minimum essential medium frozen at time zero were included in each experiment, and their values were subtracted from the experimental values to obtain the output of prostaglandins per 10^5 cells. Frozen cell suspensions were thawed, mixed well, and centrifuged (1000 × g, 10 minutes) prior to assay.

Prostaglandin assays. Prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ were measured directly from the incubation media by radioimmunoassay, with the use of procedures previously described.[15, 16] The same amount of medium was included in the assay standard curve. Preliminary experiments indicated that assaying the media directly or acidifying and extracting the media for prostaglandins prior to assay yielded the same results.

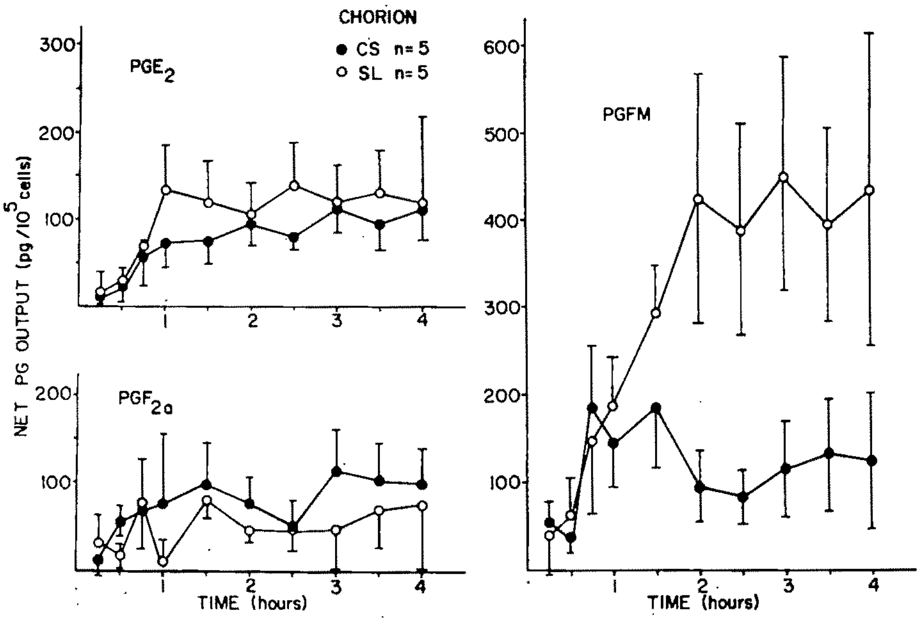

Fig. 2. The net production (mean ± SEM) of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ *(PGFM)* by dispersed cells of chorion obtained at cesarean section *(CS)* or at spontaneous vaginal delivery *(SL)*.

Results

Cell viability. Cell viability after collagenase dispersal was 89% ± 4% prior to incubation, and 77% ± 8% after 4 hours of incubation. It has been determined previously that there is no difference in cell viability between cells from tissues obtained at elective cesarean section and those obtained at spontaneous delivery.[11].

Prostaglandin production. The production of prostaglandins by amniotic cells was low before labor (Fig. 1, *CS*). However, output of prostaglandin E_2 by amnion before labor was nearly sevenfold higher than that of prostaglandin $F_{2\alpha}$ after 4 hours of incubation (682 ± 275 versus 80 ± 49 pg/ml, mean ± SEM, respectively). At labor, there was an increase in prostaglandin E_2 output, with the concentration reaching 1722 ± 484 pg/ml after 4 hours of incubation. In addition, there was a small increase in prostaglandin $F_{2\alpha}$ concentrations, to 336 ± 58 pg/ml after 4 hours. There was little production of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ by amniotic cells before or at labor.

Dispersed chorionic cells showed very low outputs of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ before labor (Fig. 2). At labor, there was no increase in the net production of either prostaglandin E_2 or prostaglandin $F_{2\alpha}$, compared to the output before labor. However, there was a marked increase in the concentration of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in the incubation media for the first 2 hours, after which there was no further net production of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$.

As in the amniotic and chorionic cell incubations, the output of prostaglandins by cells from decidua was low before labor (Fig. 3, *CS*). After 4 hours of incubation the media concentrations of prostaglandin E_2 were 239 ± 79 pg/ml, and prostaglandin $F_{2\alpha}$ and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ concentrations were 341 ± 102 and 169 ± 29 pg/ml, respectively.

At labor, production rates of all three prostaglandins increased, but the mean 4-hour output of prostaglandin $F_{2\alpha}$ was higher than of prostaglandin E_2 and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (533 ± 121 versus 370 ± 46 and 360 ± 139 pg/ml).

The ratios of the outputs of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ by amnion, chorion, and decidua are shown in Table I. Amniotic cells produced sevenfold more prostaglandin E_2 than prostaglandin $F_{2\alpha}$ before labor and at spontaneous labor. The ratios of prostaglandin E to F in the amnion were significantly higher ($p < 0.05$) than those of chorion or decidua, each of which had outputs of prostaglandin E_2 similar to those of prostaglandin $F_{2\alpha}$.

It can also be seen in Table I that the ratio of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ output was similar before and at labor in each tissue. Therefore any changes in prostaglandin production which occurred at labor resulted in little change in the relative output of prostaglandins E_2 and $F_{2\alpha}$ by any tissue.

Comment

This study describes the relative rates of production of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-di-

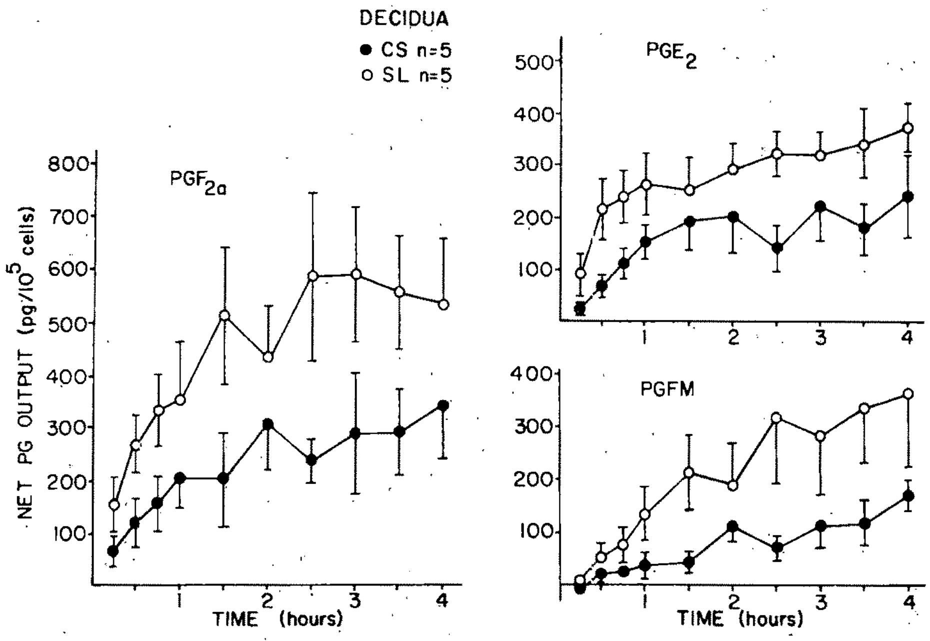

Fig. 3. The net production (mean ± SEM) of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ *(PGFM)* by dispersed cells of decidua obtained at cesarean section *(CS)* or at spontaneous vaginal delivery *(SL)*.

Table I. Ratio of net prostaglandin E_2 to prostaglandin $F_{2\alpha}$ output (mean ± SEM) in 4 hours of incubation by human amnion, chorion, and decidua cells

	Cesarean section		*Spontaneous labor*	
Cells	*n*	*Mean ± SEM*	*n*	*Mean ± SEM*
Amnion	3	6.4 ± 1.4*	5	5.9 ± 1.7*
Chorion	4	1.6 ± 1.0	3	1.2 ± 0.1
Decidua	5	0.7 ± 0.09	5	0.7 ± 0.1

*Values are significantly different from values without symbols ($p < 0.05$) as determined by unpaired t test.

hydro-15-keto-prostaglandin $F_{2\alpha}$ from endogenous precursors, by intact dispersed cells from the fetal membranes and decidua. All three tissues produced prostaglandins during the 4-hour incubation period. The production rates of the primary prostaglandins were low in amnion, chorion, and decidua obtained at cesarean section. In amnion and decidua, the output of prostaglandins E_2 and $F_{2\alpha}$ was higher at spontaneous vaginal delivery. The net output of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ did not decrease with labor in any tissue, suggesting that increases in synthesis, rather than decreases in metabolism, were responsible for the increased amount of prostaglandin $F_{2\alpha}$ in incubation media of cells obtained at spontaneous labor.

In amniotic cell incubations, the rate of appearance of prostaglandin $F_{2\alpha}$ was much slower than that of prostaglandin E_2. This difference in output was not due to metabolism of the prostaglandin $F_{2\alpha}$ to 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (Fig. 1). Other authors have also shown low rates of prostaglandin $F_{2\alpha}$ metabolism in human amnion.[8, 12] In an earlier paper,[11] we reported elevated levels of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in 6-hour amniotic cell incubations (cells obtained at cesarean section and after spontaneous labor), along with prostaglandin $F_{2\alpha}$ concentrations that were slightly higher than those shown here. However, the highest output of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ was observed in the chorionic cell incubations in both studies. It is possible that the differences in prostaglandin $F_{2\alpha}$ production and metabolism between the present study and that of Olson et al.[11] relate to minor changes in the cell dispersal procedure, the different lengths of the incubation periods in the two studies, and the small improvement in cell viability in the present study. With the present cell dispersal and incubation technique, prostaglandin E_2 is the main prostaglandin appearing in amnion cell incubations as found by Mitchell et al.[13]

In contrast, the main product of arachidonic acid metabolism found in the incubation media of chorion cells obtained at labor was 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$. The net production of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ was low before labor, and the output of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ alone increased at labor, with no elevation of prostaglandin E_2 or $F_{2\alpha}$ levels. Therefore, it appears that any prostaglandin $F_{2\alpha}$ produced by the chorion cells in vitro was rapidly metabolized to 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$. This reflects the high metabolizing activity of human chorion reported previously in homogenates.[8]

Decidual tissue obtained at spontaneous delivery produced higher amounts of prostaglandin $F_{2\alpha}$ than of prostaglandin E_2 or 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$. Although the 4-hour output of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ by decidua cells was similar to the amount measured in chorion cell incubations, it was accompanied, in decidua cells, by a parallel output of prostaglandin $F_{2\alpha}$. It has been demonstrated previously that circulating levels of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ increase at labor.[3] The relatively large amounts of prostaglandin $F_{2\alpha}$ and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ produced by decidua cells at labor may be a major source of the elevated 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in the peripheral plasma.[17]

The high levels of prostaglandins E_2 and $F_{2\alpha}$ previously reported in chorion obtained after spontaneous labor[11] and the lower levels of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ measured in decidua cell incubations in that study may be due to the difference in the technique used to separate the chorion and decidua. The "scraping" technique used in the earlier study may have resulted in a slightly different plane of separation of the two membranes with subsequent decidua cell contamination of the chorion cell incubations.

The increases in prostaglandin production that we have shown at labor do not result in any significant changes in the ratio of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ output in any tissue. Therefore, it does not appear that the increased prostaglandin production at labor is directed preferentially toward either prostaglandin E_2 or $F_{2\alpha}$. Rather, the changes in prostaglandin output are such that the relative production of the two prostaglandins remains constant before labor and at spontaneous labor.

The differences in synthetic and metabolic activities that we have found for the amnion, chorion, and decidua may be important in vivo. Casey and MacDonald[18] have suggested that the large amounts of prostaglandin E_2 produced in amnion are converted to prostaglandin $F_{2\alpha}$ in decidua, thereby providing the prostaglandin which stimulates uterine activity. Our results suggest that if prostaglandin E_2 formed in amnion enters chorion cells, then the high 15-hydroxy-prostaglandin dehydrogenase and 13,14-prostaglandin reductase activities of chorion may impede transfer of prostaglandin E_2 to decidua unless the output of the primary prostaglandin by amnion exceeds this metabolizing potential. We might speculate that precursor arachidonic acid might also cross between the membranes and decidua. Our results have also shown that decidua itself has an enhanced capacity for prostaglandin E_2 and $F_{2\alpha}$ generation from endogenous precursors at the time of labor.

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Characteristics and analysis of uterine electromyographic activity in the pregnant sheep

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Myometrial electromyographic activity was recorded with electrodes placed on the uterus in seven pregnant sheep. Electromyographic recordings were made continuously in two groups of animals. One group of four animals was studied for the first 8 days after operation at 60 to 132 days' gestation. The second group of three animals was studied at least 8 days after operation. Myometrial electromyographic activity could be divided into epochs of varying duration. With the use of microprocessor-based data acquisition systems and computerized analysis it was demonstrated that (1) on the basis of an analysis of duration of electromyographic activity there were at least two populations of electromyographic epoch, (2) myometrial electromyographic activity increased in the first 3 to 4 days after operation, and (3) by 6 to 7 days after operation a relatively stable level of activity had been achieved. The separation of electromyographic activity into two populations was most marked in the animals observed in the immediate postsurgical period. In these animals the population of epochs with the longer-lasting activity had a median duration of 5.9 minutes in eight pairs of electromyographic electrodes from four animals. Curve-fitting techniques demonstrated that no more than 14% of uterine electromyographic activity had a duration <3 minutes. The longer-lasting group of myometrial electromyographic events corresponds to uterine contractures we have described previously; the other group of epochs of activity was of shorter duration. (AM J OBSTET GYNECOL 1985;151:524-31.)

Key words: Uterine electromyographic activity, pregnancy, myometrium

Myometrial activity throughout pregnancy has been described in several species including the sheep[1-5] and monkey.[6-9] The characteristics of this activity, its origin, the mechanisms responsible for its regulation, and the physiologic significance of the different types of myometrial activity have recently received considerable attention.[2, 10-13] Direct visual analysis of recordings of electromyographic activity in the pregnant sheep in late gestation demonstrated bursts of myometrial electromyographic activity lasting different lengths of time.[3-5, 10]

Myometrial contractions such as those that occur at labor and delivery produce increases of intrauterine pressure that last about 1 minute. The electromyographic activity associated with these contractions usually lasts <1 minute. In addition to these short-lived bursts of activity, we have described epochs of myometrial electromyographic activity that last longer than 4 minutes and are associated with low-amplitude increases in intrauterine pressure. The term "contractures" has been used to distinguish these episodes from labor and delivery contractions.[1-3] They have previously been described on the basis of both intrauterine pressure changes[1-3] and electromyography.[3] Both of these indices of myometrial activity are important. However, electromyography probably provides a more direct index of the drive to myometrial function, whereas intrauterine pressure changes also reflect the mechanical properties of the supporting tissues of the uterus.

Visual analysis of electromyographic records is an important first step to the experimental interpretation of changes in myometrial activity. However, simple visual analysis may not provide adequate quantification of basal myometrial activity. In addition precise techniques are required to analyze qualitative alterations in contracture and contraction activity in response to different situations. The frequency distribution of myometrial electromyographic epochs of varying duration in individual animals has not previously been determined. The aims of the present study were (1) to develop a precise quantitative method for analysis of myometrial electromyographic epochs, (2) to observe the frequency distribution of electromyographic epochs of different duration in order to determine whether contractures constitute a distinct, homogeneous population of events, and (3) to determine how the amount of myometrial activity changes in the immediate postsurgical period.

Methods

Care of animals. Three Rambouillet-Columbia and four Dorset ewes of known gestational age were used

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in this study. Pairs of myometrial electromyographic electrodes were placed as described previously.[3] In ewes carrying a single fetus, the electromyographic electrodes were always placed on the pregnant horn. In addition, the uterus was incised for placement of fetal vascular catheters by means of techniques that we have described in detail.[2, 3, 14] The day of operation was considered day zero. The four Dorset ewes were studied in the 8 days immediately after operation (group 1). The three Rambouillet-Columbia ewes were studied at least 8 days after operation (group 2).

Recording of uterine electromyogram, digitization of signal, data storage, and calculation of total myometrial activity and frequency of bursts of different durations

Data acquisition and storage. The uterine electromyogram was recorded from multistranded bipolar stainless steel wires (Cooner AS 632) implanted 3 to 5 mm apart in the myometrium. They were placed on at least two sites of the myometrium as previously described.[2, 15] As far as possible, the recording portion of the wire was sewn through all the muscular layers of the uterus. The electromyographic signal was amplified (200 μV full-scale deflection), band-pass filtered (3 to 20 Hz), full-wave rectified, and low-pass filtered (1 Hz) to create the envelope of the electromyographic bursts. Each variable was sampled 32 times per second with 8 bit accuracy or 0.4% of full-scale deflection and the readings accumulated. At 8-second intervals, the mean activity for each channel during that interval was computed from the accumulated values and transmitted, on a high-speed serial digital line, to an Apple II–based data collection and storage system in a central monitoring room. The integrated uterine electromyographic activity is represented as units where one unit is 6.25 μV · sec. The signals integrated over 8 seconds were stored on a floppy disk together with a time signal. To provide immediate display, the uterine electromyographic tracing was also recorded on a Beckman 711 pen-recorder. This record of activity was also used to verify the computer-based calculations. The initial sampling frequency of 32 Hz prevents aliasing problems with changing signals, while the lower storage rate of one reading every 8 seconds creates a manageable volume of data for subsequent analysis.

Computer analysis of total myometrial electromyographic activity and duration of individual myometrial epochs. A computer program was written to analyze the myometrial electromyographic data. The initial problem was to define a signal/noise threshold. This was initially performed by eye since, as previously described by ourselves and others, the electromyographic signals produced by contractures are clearly differentiated.[3-5] In the computerized analysis, a signal/noise threshold was defined by summing data points over a 2-hour period and calculating the mean. Then each new data point was considered "real activity" if it exceeded this threshold. With each new point, the threshold was updated by adding this point to the sum, subtracting the point 2 hours before, and recalculating the mean. This procedure was repeated throughout the data. Movement of the mean used for calculating the signal threshold with the individual signal being evaluated allowed for changes in background activity.

In a previous publication, a contracture was defined to be an epoch of continuous uterine electromyographic activity that lasted at least 4 minutes and exceeded the minimum threshold as determined from direct examination of the pen-recording.[16] Our intention in the present study was to obtain more precise data on the frequency distribution of electromyographic bursts of all lengths. A major problem in the analysis of the frequency distribution of different myometrial electromyographic epochs of different duration was to define the termination of an epoch. In our basic analysis, if the data fell below the signal/noise threshold (subthreshold) for longer than 16 seconds, that particular myometrial electromyographic epoch was considered to have terminated. We have called the period of subthreshold electromyographic activity required to terminate an epoch, the delimiter. The output from the program consisted of time duration of each epoch, integrated electromyographic activity during that epoch, and elapsed time of no activity between epochs.

Curve-fitting procedures to determine the presence of different populations of electromyographic epochs of different durations. It was desired to derive equations that would describe the frequency distribution of epochs of electromyographic activity of different durations.

From an initial analysis of the data from sheep in group 1 it seemed that the observed data for the number of epochs of different durations recorded in a given period could be represented by the linear sum of a Gaussian model for the longer-duration epochs and a decay model for the population of shorter-duration epochs. Data were separated in the following manner. The second peak in the data (the peak that occurred at $t > 1$ minute) was selected as the peak time of the Gaussian model. The assumption was made that the contribution by the decay model was insignificant at times equal to and greater than time of this second peak (T). With the use of the property of symmetry about the peak in Gaussian models, theoretical data for the model at times before the peak time (T) were obtained.[17]

The data for the decay model were obtained by subtracting theoretical data generated by the Gaussian model from the original data at corresponding times.

For the Gaussian model the following equation was used to derive L_i at each value of t_i greater than the peak of the Gaussian distribution[17, 18]:

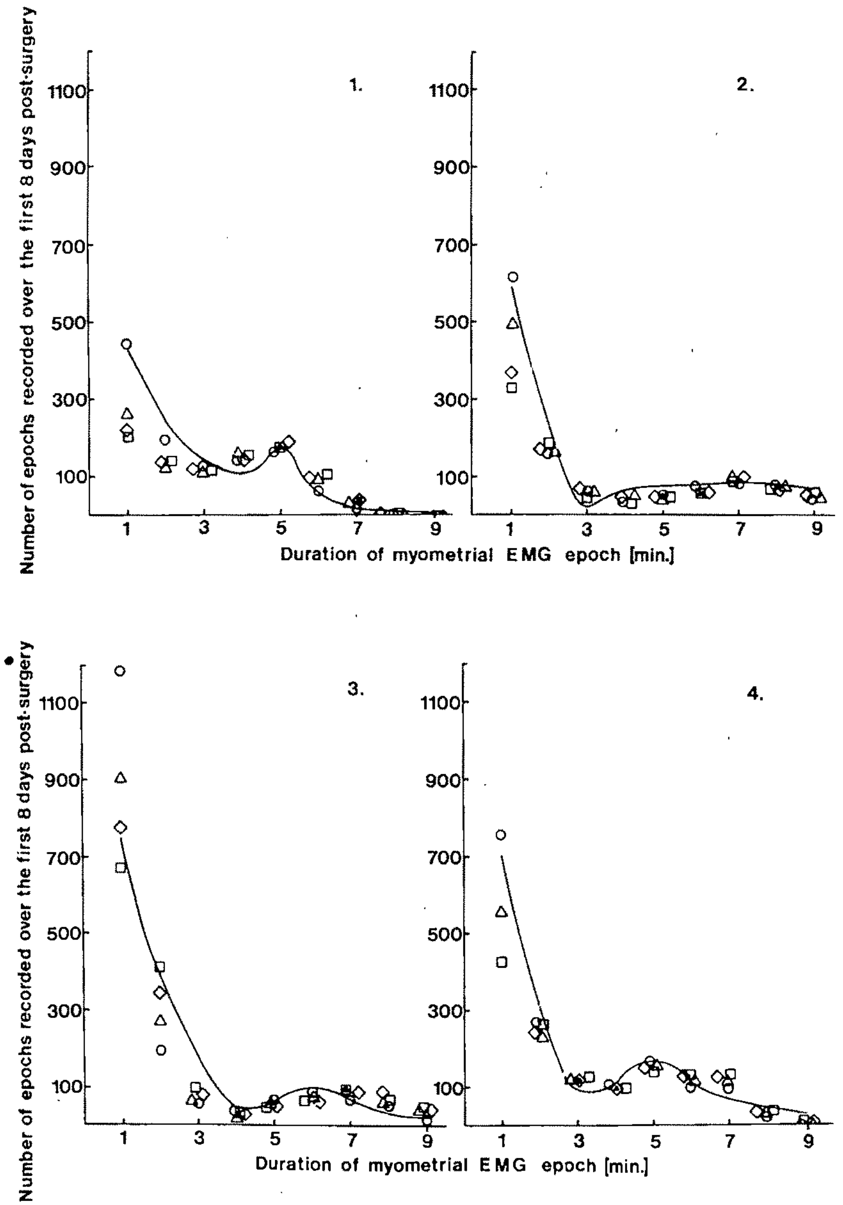

Fig. 1. Frequency distribution of myometrial electromyographic epochs of different durations are shown in four animals during 0 to 7 days (the first 8 days) after operation. Day of operation = day 0 (group 1). The effect of changing the delimiter, the required duration of electromyographic quiescence to terminate an epoch for the purposes of calculating the frequency distribution of different lengths of electromyographic epochs, is also shown. Various delimiters were used: 16 seconds of quiescence (○), 24 seconds of quiescence (△), 32 seconds of quiescence (◇), 40 seconds of quiescence (□). Sheep numbers correspond to the numbers in Table I. The *line* is the best-fit line calculated for the 16-second delimiter.

$$L_i = \frac{1}{2}\left(\frac{-(t_i - T)}{\log e\frac{Y_i}{A}}\right) \qquad (1)$$

where Y_i = frequency of epochs in each time interval (i), A = frequency of epochs at peak of the Gaussian curve, T = duration of epochs at peak of the Gaussian distribution (length of most frequent epoch), and $t_i \doteq$ time interval corresponding to Y_i.

When all L_i values were obtained, $\bar{L}$ (which estimates the width of the distribution curve) was calculated to predict the values at any t_i with the following equation:

$$G_i = A \cdot e \frac{-(t_i - T)}{2\bar{L}} \qquad (2)$$

where G_i is the predicted frequency of epochs at each t_i.

Theoretical values from the Gaussian model were sub-

Table I. Values obtained from the decay and Gaussian curve-fitting equations

Electromyographic electrode	*B*	*C*	*A*	$\bar{L}$	*T*	*Location of leads*	*No. of fetuses*
Sheep 1							
1	266	422	168	0.43	5	PH1	t
2	215	246	150	1.85	4	PH2	t
Sheep 2							
1	191	303	76	2.17	7	PH	s
2	163	278	66	3.13	7	Body	s
Sheep 3							
1	527	739	99	1.02	6	PH	s
2	524	815	68	1.22	7	PH	s
Sheep 4							
1	227	351	110	0.72	6	Body	s
2	267	331	109	1.14	5	Body	s

The composite myometrial electromyographic data from two pairs of electrodes on each pregnant ewe were analyzed for the first 8 days after operation. PH = Pregnant horn. B is the rate of decay of the exponential model fitted to the epochs of shorter duration; C is the theoretical frequency of short-duration epochs at time 0; A is the frequency of longer-duration epochs at the peak of the Gaussian curve; $\bar{L}$ is an estimate of the width of the Gaussian curve; T is the time at the peak of the Gaussian curve. s = Single fetus; t = twins.

tracted from real data at each t_i and the remaining activity at each t_i fitted to the decay model.

The equation for the decay model is of the form:

$$D_i = -B \cdot \log_e t_i + C \qquad (3)$$

where B is the decay rate and C is the frequency of epochs at $t_i = 0$.

The linear sum of equations 2 and 3 was used to predict the frequency of epochs at any time t_i under the assumption that at t_i greater than T the contribution of the decay model is negligible and takes the form:

$$F_i = G_i + D_i$$

where F_i = theoretical number of epochs at time t_i.

Statistical analysis. Analysis of variance or the one-tailed Student's *t* test with the Bonferroni transformation for multiple comparisons was used for all analyses.[18] Whenever multiple comparisons between means were performed, the Bonferonni transformation was used unless otherwise indicated.

Results

Outcome of experimental preparations. Four of the seven ewes delivered live fetuses at term. The remaining three pregnancies were used for alternative experiments after completion of the electromyographic recordings. Fetal blood gas values were within the normal range[14] at the conclusion of uterine electromyographic recording. Three ewes were carrying twin lambs and four ewes had single fetuses.

Effect of altering definition of the delimiter that terminates an epoch. The use of a 16-second period of electromyographic activity below the calculated 2-hour mean threshold as the delimiter of activity was an arbitrary definition. It was chosen as a result of scanning many hundreds of hours of myometrial electromyographic activity obtained from more than 30 pregnant sheep at various stages of pregnancy during the last 90 days of gestation. It was possible that some of the electromyographic epochs contained, within themselves, short periods of subthreshold electromyographic activity and hence distorted the analysis. If this were true, lengthening the period of subthreshold activity necessary to qualify as the delimiter of an epoch should decrease the total number of epochs, especially those of shorter duration. To investigate this possibility we analyzed the effect of increasing the duration of the delimiter. We wished to see whether changing the duration of the delimiter would alter the frequency distribution of different lengths of electromyographic epochs (Fig. 1). It can be seen that there was very little change in the number of epochs with a duration longer than 3 minutes when the delimiter was increased from 16 to 40 seconds. As would be expected there was a drop in the shorter-lived epochs as these were merged together by this form of analysis. In view of the small effect observed of changing the delimiter from 16 seconds to as much as 40 seconds on the occurrence of the longer-duration "contracture" events, we accepted the initial visual observation that a 16-second delimiter was adequate as the delimiter of an electromyographic epoch.

Curve fitting to demonstrate different populations of different epoch durations. The composite frequency distribution curves for epochs of continuous myometrial activity of different durations over the full 8 days in each animal in group I are shown for all four animals in Fig. 1. This figure shows both the raw data and the theoretical curve for one pair of electromyographic electrodes from each animal. The curve-fitting regimen used attempts to separate the epochs of myometrial activity into two populations of events in each ewe. The

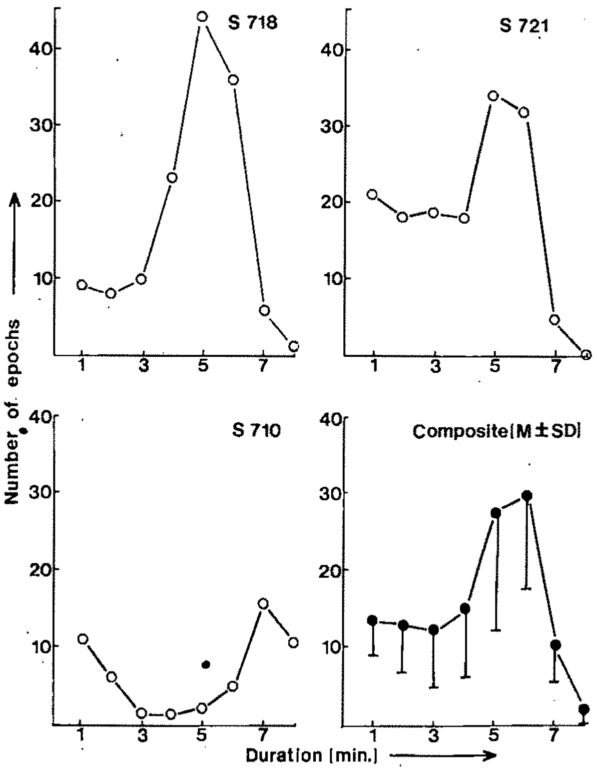

Fig. 2. Distribution of epochs of different durations (delimiter of 16 seconds) in three animals at 114 to 140 days' gestational age and at least 8 days after operation in one 24-hour period. The composite figure for the mean ± SD for the three animals is also shown.

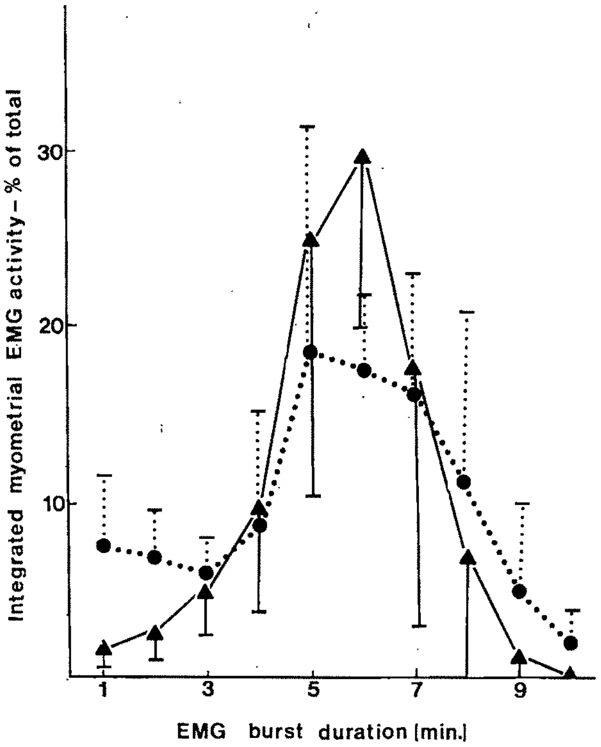

Fig. 3. Percentage of total activity (mean ± SD) in each 1-minute group of myometrial electromyographic burst duration in four sheep during the first 8 days after operation at 60 to 132 days' gestation, group 1 (● · · · ●) and in three pregnant ewes at least 8 days after operation at 114 to 140 days of gestation, group 2 (▲——▲).

values for the unknowns in the equation described in the Methods section are given in Table I. It can be seen that the correlation in the two leads in the same animal was good for all four animals. In addition, parameters in the equation for the one twin pregnancy did not differ from those of the three ewes carrying a single fetus.

Frequency distribution curves for epochs of continuous myometrial activity of different durations in three pregnancies over one full 24-hour period at 114 to 140 days' gestation and at least 8 days after operation (group 2) are shown in Fig. 2. When compared with electromyography in the animals studied during the first 8 days after operation and represented in Fig. 1, uterine electromyography in animals studied at least 8 days after operation showed little activity in the epochs of short duration.

Integrated myometrial activity. Fig. 3 compares the total amount of electromyographic activity that occurred in the electromyographic epochs of different durations. In the four sheep in the immediate postoperative period, it can be seen that 25% of the activity existed in epochs lasting ≤3 minutes and 31% in epochs lasting ≤4 minutes. In the three animals studied at least 8 days after operation, even less of the activity (14%) occurred in epochs lasting ≤3 minutes and 17% in epochs lasting ≤4 minutes. These small amounts of activity of short-duration epochs support our previous definition in the sheep of a contracture as an epoch of uterine electromyographic activity lasting at least 4 minutes.[16] With the use of analysis of variance in both populations it can be shown that the distribution of activity of different durations is not a uniform distribution ($p \leq 0.0001$). Furthermore, in both of these groups a Gaussian distribution can be fitted to the data with peaks at 5 and 6 minutes. The total amount of electromyographic activity changed in the postsurgical period (Fig. 4). Comparison of the two groups of animals demonstrates that there was relatively more short-lived activity in group 1 animals studied in the early days after operation, measured as the percentage of total electromyographic activity (Fig. 3) (7.5 ± 4.2 for events lasting ≤1 minute and 7.0 ± 2.5 for events lasting ≤2 minutes), than in the animals in group 2 that were studied at least 8 days after operation (1.6 ± 0.8 for events lasting ≤1 minute, $p < 0.001$, and 2.6 ± 1.5 for events lasting ≤2 minutes, $p < 0.001$, Student's *t* test for nonpaired observations).

Integrated uterine electromyographic activity recorded in 10 pairs of electrodes in the four animals studied in the immediate postoperative period (group 1) is shown in Fig. 4. All myometrial electromyographic activity above baseline and lasting >3 minutes was summed for two 12-hour periods each day, midnight to noon and noon to midnight. Activity over 12-hour periods in each pair of electrodes was normalized so that maximal activity in any 12-hour period was 100% regardless of the day on which that maximum activity occurred. The present form of analysis is inappropriate for the study of circadian rhythmicity for two reasons. First, an underlying circadian rhythm may have been masked by an evolving change in myometrial activity over the days immediately after operation. Future experiments to determine whether circadian rhythmicity is present will require observation of animals after the onset of the stable phase of electromyographic activity shown in Fig. 4, that is, at least 8 days after operation. Second, it will be necessary to sum periods of activity more frequently than every 12 hours. Analysis of variance and multiple comparisons of the data shown in Fig. 4 demonstrated several statistically significant features of the integrated myometrial activity in the 8 days after operation. Electromyographic activity increased after operation and by 48 to 60 hours it was higher than in the period 12 to 24 hours after operation ($p < 0.05$). All values thereafter were statistically higher than in the first recording period immediately after operation ($p < 0.05$). The fall from day 5 to the low values on days 6 to 8 was not statistically significant when the Bonferroni transformation was applied. However, with a simple one-tailed *t* test the difference was significant, $p < 0.01$. Integrated electromyographic activity seemed to have stabilized by day 6 after operation.

Comment

Myometrial contractions during labor and delivery have been intensively investigated in an attempt to understand the mechanism of parturition. The demonstration of a different form of myometrial activity, contractures, over a major portion of gestation has raised at least three major questions. First, what factors initiate and regulate contractures? Second, what is the relationship of contractures to contractions? Are they the same entity but of different duration, or does one give way to the other? Third, what is the relationship between contractures and the related changes in fetal behavioral state?[2, 3, 11, 12, 16] A precise answer to these questions requires a firm quantitative description of myometrial activity based on long-term in vivo recording. Any useful analysis should provide a few concise terms to describe the pattern of electromyographic activity so that meaningful and statistically acceptable comparisons between different electromyographic recordings can be made. If possible, these terms should be insensitive to the absolute amplitude of the electromyographic signal and other variables that depend on electrode characteristics.

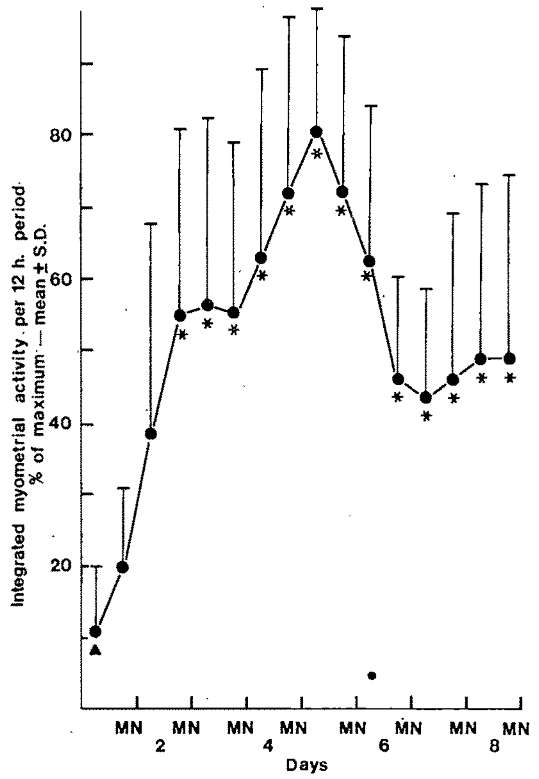

Fig. 4. Integrated myometrial activity contained in epochs lasting at least 3 minutes. Data from four pregnant ewes recorded at 60 to 128 days' gestation. Day of operation is day 0. *MN* = Midnight. Data were obtained from 10 pairs of electromyographic electrodes at various sites on the uterus, including the body and the horns, in the four animals and are plotted as mean ± SD. Data collection began at midnight on the night after operation. Activity in each lead was integrated during the 12-hour periods midnight to noon and noon to midnight. Activity during 12-hour periods in each lead was normalized so that maximal activity in any 12-hour period was 100% regardless of the day on which that maximum activity occurred. Points marked with an *asterisk* are significantly different from the immediate postoperative value (▲) by Student's *t* test with Bonferroni transformation for multiple comparison.

We and other investigators have previously divided myometrial electromyographic epochs into arbitrary groups of differing durations.[3, 5] The present method of analysis is an extension of this form of discontinuous categorization. It has demonstrated that there is a continuous spectrum of duration of different epochs and that these events can be divided mathematically into two populations. The epochs of longer duration correspond to the events previously referred to as contractures. In the absence of intrauterine pressure recordings we are unable to comment on the relationship of the shorter-duration epochs to labor and delivery contractions. In other studies in which we have recorded intrauterine pressure many days prior to labor and delivery, intrauterine pressure changes resembling labor and delivery contractions were not observed. It may be that the short-lived epochs cannot produce labor and delivery–type intrauterine pressure changes because of the absence of the correct hormonal milieu at this time.

Knowledge of the frequency distribution of the different myometrial epochs helps to answer related questions: Are contractures really a discrete form of myometrial activity? What are their significant characteristics? If they are a discrete population, what are the time characteristics that define a contracture? The curve-splitting technique used allows us to make some comment as to valid criteria for choosing different epoch durations. Such definition is required to analyze the effect of different agents and perturbations (for instance, operation and various drugs). On the basis of our present analysis we would be prepared to define contractures as periods of continuous myometrial electromyographic activity lasting at least 3 minutes that do not contain more than 16 seconds of subthreshold activity.

In experiments relating to myometrial or fetal physiologic characteristics in the chronically instrumented pregnant sheep, the uterus is generally incised in one or more locations. The sequence of changes in materrial and fetal physiologic condition in the immediate postoperative period has been studied in relation to several different systems. Considerable attention has been focused on the time interval after operation that is necessary to attain postoperative stability of different systems. In order to elucidate the possible postoperative effects of endocrine and other systems on myometrial electromyographic activity it is necessary to describe both the amount and the pattern of postoperative activity.

It has been demonstrated that maternal prostaglandin concentrations are elevated after operation.[19] A role for prostaglandins in the control of contractures is further supported by our observation that both antipyrine (unpublished data) and 4-aminoantipyrine administered to either the pregnant ewe or her fetus will diminish the frequency and total amount of contracture activity. The doses of these agents that affected contractures also lowered maternal 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ metabolite concentrations.[20] Thus prostaglandins appear to play a role in the control of contractures. The related question as to the hormonal or neuronal mechanisms responsible for the termination of a contracture remains to be elucidated.

The postoperative increase in total integrated myometrial activity of >3 minutes' duration observed in the present study requires consideration. As discussed above it is possible that after operation the dominant drive to the uterus may produce more short-lived contraction–type activity. Alternatively, after operation there may be a gradual increase in some stimulatory factor during the first few days resulting in a gradual increase and eventual stabilization of activity at an elevated level, which may be above that in the noninstrumented animal. Another possibility is that operation may stimulate a net inhibitory effect on contractures with an initial decrease in myometrial activity below the preoperative uninstrumented level. Further experiments, such as repeat operations on animals already bearing uterine electromyographic electrodes, will be required to answer these questions. Delineation of other factors such as diurnal rhythmicity and subtle relationships of fetal activity to contractures will require long-term study with data acquisition and analytical systems such as described here.

We would like to thank Kathryn Fontwit, Angela Massman, and Robert Johnson for their assistance with these experiments.

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Fetal breathing movements and lung hypoplasia: Preliminary human observations

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Hypoplasia of the fetal lung is found in association with long-standing oligohydramnios. In animal models, interference with fetal breathing activity has led to lung hypoplasia. It has been suggested that lung hypoplasia associated with oligohydramnios is due to inhibition of fetal breathing. Observations of seven patients with prolonged oligohydramnios, three of whom had lung hypoplasia, indicate that fetal breathing does occur in these cases and that it is unlikely that lung hypoplasia is merely the result of absent fetal breathing activity. In fact, when compared to the four patients with oligohydramnios and presumably normal lungs, the three patients with oligohydramnios and lung hypoplasia spent more time breathing and did so at higher rates. (AM J OBSTET GYNECOL 1985;151:531-3.)

Key words: Fetus, amniotic fluid, fetal breathing, oligohydramnios, lung hypoplasia

Hypoplasia of the fetal lungs is found in association with long-standing oligohydramnios. Potter[1] first reported on a series of babies with renal agenesis and noted that lung hypoplasia was a constant feature. It was later recognized that the same phenotype, including lung hypoplasia, could be seen in newborn infants with posterior urethral valves, in the donors of severe and chronic twin-to-twin transfusion, or simply following prolonged leakage of amniotic fluid.[2-4] In all of these cases, oligohydramnios was present for an extended period of time. Nimrod et al.[5] have recently reported a 9% incidence of lung hypoplasia following very prolonged rupture of the membranes. Experimental production of oligohydramnios has led to lung hypoplasia in several animal species.[6-9]

The pathogenesis of the pulmonary hypoplasia associated with oligohydramnios is unclear. It has been speculated that hypoplasia of the lungs in this situation is the result of fetal compression (either thoracic or abdominal) leading to reduced space available for lung growth.[2, 10, 11] More specifically, it has been proposed that oligohydramnios could enhance the outflow of fetal lung liquid and thus interfere with lung expansion in utero.[11-13] Both experimental tracheal ligation and drainage of fetal lung liquid, by resulting, respectively, in hyperplasia and hypoplasia of the lung, support the concept that lung expansion in utero is an important determinant of fetal lung growth.[14]

Fetal breathing movements, by intermittently raising

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Table I. Clinical findings in three cases of lung hypoplasia

Case No.	*Gestational age (at study session) (wk)*	*Membrane status (gestational age at rupture)*	*Outcome*	*Autopsy findings*
010	28-34 (5 studies)	Intact	Respiratory failure, death <24 hr	Renal agenesis Lung hypoplasia* Amnion nodosum of placenta
012	37	Intact	Respiratory failure, death <24 hr	Permit not granted on religious grounds Polycystic kidneys with lung hypoplasia* Placenta not available
020	27	24 wk	Respiratory failure, death <24 hr	Lung hypoplasia* Hyaline membrane disease Amnion nodosum of placenta

*The diagnosis of lung hypoplasia was based on (1) lung weight <2 SD below the norm for Case 010; (2) clinical course and known pattern of associated malformations for Case 012; (3) biochemical (DNA) and histologic criteria for Case 020 (reference 9).

Table II. Fetal breathing activity: Infants with oligohydramnios versus control infants

Infants	*Proportion of time spent breathing (mean ± SD)*	*p*	*Rate of breathing, breaths/min (mean ± SD)*	*p*
Oligohydramnios ≥3 wk (n = 7)	0.09 ± 0.10	0.246	27.5 ± 15.1	0.184
Control (n = 7)	0.14 ± 0.14		34.3 ± 11.9	

Table III. Fetal breathing activity following oligohydramnios: Comparison of fetuses with or without lung hypoplasia

	Proportion of time spent breathing (mean ± SD)	*p*	*Rate of breathing, breaths/min (mean ± SD)*	*p*
Oligohydramnios and lung hypoplasia (n = 3)	0.18 ± 0.10	0.014	36.5 ± 10.2	0.011
Oligohydramnios and normal lungs (n = 4)	0.02 ± 0.01		16.0 ± 6.4	

airway distending pressure, have been held responsible for contributing to normal fetal lung growth. Experimental division of the neural pathways supplying the respiratory system (bilateral phrenectomy or cervical cord section) and fetal paralysis (induced with curare) have led to lung hypoplasia.[15-17] Both Gruenwald[10] and Wigglesworth[18] have postulated that failure of lung growth in association with oligohydramnios could be due to inhibition of breathing movements.

The purpose of this report is to present an analysis of fetal breathing movements in seven patients with oligohydramnios resulting in lung hypoplasia in three of the seven cases.

Patients and methods

Seven patients with prolonged oligohydramnios (two with intact membranes and renal anomalies, five with prolonged spontaneous rupture of the membranes for ≥3 weeks) were studied in a total of 14 1-hour sessions at gestational ages ranging from 27 to 38 weeks. Session by session, a gestational age–matched control subject with intact membranes and normal amniotic fluid volume was identified and observed in an identical fashion. Fetal breathing activity was recorded by longitudinal fetal axis linear-array B-mode sonography as previously described,[19] and analyses of the proportion of observation time spent in fetal breathing activity and of breathing rate during breathing intervals (breaths per minute) were carried out. All sessions occurred at the same time of the day and approximately 2 hours after a meal of about 350 kcal. No patient smoked within 4 hours of the recording session, and none had evidence of hypertension, abnormal carbohydrate metabolism, or clinical amnionitis. Oligohydramnios was defined by ultrasound assessment at each study session and was confirmed at the time of delivery.

Of the seven cases of oligohydramnios, four infants died in the early neonatal period. Three of those were considered to have lung hypoplasia and their clinical findings are summarized in Table I. At autopsy the lungs of the infant in the fourth case weighed less than expected for gestational age and body weight but did not meet the criteria for the diagnosis of lung hypoplasia.[11] Placental tissue was available in four of the seven cases (one infant was delivered at another institution) for a retrospective evaluation of the amnion by light microscopy. Amnion nodosum, a lesion thought to be pathognomonic of prolonged oligohydramnios, was seen in three of the four placentas available for study.[2]

We recorded 14 study sessions in seven patients with oligohydramnios and 14 sessions in gestational age–matched control subjects. When a patient was studied more than once, only the mean value of the results was used for comparison. We compared the incidence and the rate of fetal breathing movements in the seven patients with prolonged oligohydramnios and in their matched control subjects by paired sample analysis. We also compared the same parameters in two subsets of the oligohydramnios group by the two-sample t test, that is, the three patients with lung hypoplasia were compared to the four patients without lung hypoplasia.

Results

Fetal breathing activity was present in all seven cases of prolonged oligohydramnios. When compared to the matched control fetuses with normal amniotic fluid volume, the fetuses with oligohydramnios, taken as a group, tended to spend less time breathing and did so at lower rates. These apparent differences in the means of results did not however reach a level of statistical significance (see Table II). When compared to fetuses with oligohydramnios but normal lung growth, fetuses with oligohydramnios and lung hypoplasia (at the time of delivery) spent significantly more time breathing and did so at significantly higher rates (see Table III).

Comment

All seven fetuses with prolonged oligohydramnios studied by linear-array B-mode sonography did show evidence of fetal breathing activity. Neither the incidence nor the rate of fetal breathing differed between these patients and their matched control subjects with normal amniotic fluid volume. Our recent experience with an animal model of amniotic fluid drainage led to similar findings. We found no difference in the incidence of fetal breathing movements in fetal lambs when comparing a 24-hour period preceding and a same period immediately following amniotic fluid drainage.[9] We conclude that it is unlikely that the lung hypoplasia seen with oligohydramnios is merely the result of the absence of fetal breathing movements. Whether other characteristics of fetal breathing, such as the transient alterations in lung volume,[20, 21] are modified by oligohydramnios is unknown at this point.

The observation that fetuses with both oligohydramnios and lung hypoplasia, when compared to fetuses with oligohydramnios and normal lungs, actually spent more time breathing and did so at higher rates was unexpected and is challenging. Whether this phenomenon relates to lung hypoplasia as a cause or as a consequence is unclear. However, and beyond that last question, it would be interesting to evaluate prospectively whether high rates and/or incidences of fetal breathing could become useful clinical indicators of lung hypoplasia in association with oligohydramnios.

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Estradiol potentiation of gonadotropin-releasing hormone responsiveness in the anterior pituitary is mediated by an increase in gonadotropin-releasing hormone receptors

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In order to investigate the mechanism by which 17β-estradiol potentiates the action of gonadotropin-releasing hormone on the anterior pituitary in vitro, cultured pituitary cells from immature female rats were used as the model system. Cultures exposed to estradiol at concentrations ranging from 10^{-10} to 10^{-6} mol/L exhibited a significant augmentation of luteinizing hormone release in response to a 4-hour gonadotropin-releasing hormone (10 μmol/L) challenge at a dose of 10^{-9} mol/L compared to that of control cultures. The estradiol augmentation of luteinizing hormone release was also dependent on the duration of estradiol exposure. When these cultures were incubated with tritium-labeled L-leucine, an increase in incorporation of radiolabeled amino acid into total proteins greater than that in controls was observed. A parallel stimulatory effect of estradiol on iodine 125–labeled D-Ala^6 gonadotropin-releasing hormone binding was observed. Cultures incubated with estradiol at different concentrations and various lengths of time showed a significant increase in gonadotropin-releasing hormone binding capacity and this increase was abrogated by cycloheximide. Analysis of the binding data showed that the increase in gonadotropin-releasing hormone binding activity was due to a change in the number of gonadotropin-releasing hormone binding sites rather than a change in the affinity. These results suggest that (1) estradiol treatment increases the number of pituitary receptors for gonadotropin-releasing hormone, (2) the augmentary effect of estradiol on luteinizing hormone release at the pituitary level might be mediated, at least in part, by the increase in the number of binding sites of gonadotropin-releasing hormone, and (3) new protein synthesis may be involved in estradiol-mediated gonadotropin-releasing hormone receptor induction. (AM J OBSTET GYNECOL 1985;151:534-40.)

Key words: Gonadotropin-releasing hormone receptor, estradiol modulation, pituitary cell culture, luteinizing hormone

The positive-feedback mechanism of estrogens on the regulation of pituitary gonadotropins in the human is well established. Brown et al.[1] have shown evidence of ovulation in some anovulatory women after injection of estrogens. Swerdloff and Odell[2] and Vande Wiele et al.[3] have shown an increase in serum luteinizing hormone 36 hours after intravenous administration of Premarin. An increase in luteinizing hormone and follicle-stimulating hormone following administration of progesterone has been demonstrated in postmenopausal women by Odell and Swerdloff.[4] Similarly, Yen and Tsai[5] and Monroe et al.[6] have also shown an increase in luteinizing hormone following administration of estradiol in the early follicular phase. Thus an overwhelming body of evidence exists in favor of an augmentary role of estradiol on luteinizing hormone release. These effects have been shown to result from steroid action both at the hypothalamic level and at the hypophyseal level.[7]

In order to elucidate the cellular mechanisms underlying the augmentary effect of estradiol in modulating the pituitary response to gonadotropin-releasing hormone (GnRH), we have used an in vitro series of studies with enzymatically dispersed rat pituitary cells grown in primary monolayer culture as a model system. In the rat, in vitro studies have shown a direct estradiol effect on the responsiveness of the pituitary for luteinizing hormone release in response to GnRH.[8, 9] It has also been shown that there is a correlation between in vivo estrogen levels and the number of pituitary GnRH receptors.[10, 11] The present studies were done to determine whether estradiol acts directly at the pituitary level to cause a potentiation of GnRH responsiveness with respect to luteinizing hormone release. In addition, the effect of estradiol pretreatment on GnRH receptors in cultured pituitary cells was examined to determine whether estradiol acts by increasing the number of GnRH receptors. Our results show that es-

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tradiol pretreatment potentiated GnRH-stimulated release of luteinizing hormone from the pituitary cell cultures in a concentration- and time-dependent manner. Furthermore, the potentiating effect of estradiol on release of luteinizing hormone at the pituitary level might be mediated, at least in part, by an increase in the number of GnRH receptor sites in the pituitary, and the evidence suggests that synthesis of new protein may be involved in this process.

Material and methods

Female Sprague-Dawley rats, 21 days old, were purchased from Spartan Farms, Haslett, Michigan, for use in these studies. Synthetic GnRH was a gift from Parke-Davis, Ann Arbor, Michigan, and D-Ala^6 des-Gly^{10}-N-ethylamide GnRH (D-Ala^6 GnRH) was purchased from Beckman Instruments–Spinco Div., Palo Alto, California. Estradiol was purchased from Sigma Chemical Co., St. Louis, Missouri. Reagents for cultures were obtained from Grand Island Biological Co. (GIBCO), Grand Island, New York; Worthington Biochemical Corp., Freehold, New Jersey; and Sigma Chemical Co. Tritium-labeled L-leucine (specific activity 110 Ci/mmol) was purchased from New England Nuclear, Boston, Massachusetts. Reagents for radioimmunoassay were provided by the Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD).

Preparation of pituitary monolayer cultures. Anterior pituitaries were obtained from immature female Sprague-Dawley rats. The cell cultures were prepared by a previously described procedure[12] with some modifications. Anterior pituitaries were minced and incubated in calcium and magnesium-free Earle's balanced salt solution containing 0.3% collagenase, 0.15% hyaluronidase, and 2% bovine serum albumin for 90 minutes, followed by 0.25% pancreatin for 15 minutes. After centrifugation and washing of the material, the dispersed cells were counted, divided into aliquots (10^6 cells per 2 ml) to tissue culture plates (35 × 10 mm, Falcon Plastics, Los Angeles, California), and maintained at 37° C in a water-saturated atmosphere of 95% air and 5% carbon dioxide. The medium used for culture was Dulbecco's modified Eagle's medium supplemented with 10% horse serum, 2.5% fetal calf serum, nonessential amino acids, glutamine, 50 ng/ml of gentamicin, and 10 IU/ml of Mycostatin. The sera were previously treated with dextran-coated charcoal to adsorb the endogenous steroids. Radioimmunoassay of charcoal-adsorbed sera indicated nondetectable levels of estradiol.

Incubation procedure. After cell attachment, which usually takes place in 48 hours, the medium was replaced with fresh medium, and experimental treatments were initiated. Estradiol was dissolved in 70% ethanol, and 10 μL of this ethanolic solution was added to the cultures to achieve the final desired concentration. Control cultures received an equivalent volume of diluent alone. After incubation for indicated times with the test substances, cultures were washed and challenged for 4 hours in serum-free Dulbecco's modified Eagle's medium containing 10^{-8} mol/L of GnRH. At the end of this incubation period, media were separated from the cells and luteinizing hormone was assayed in the media by radioimmunoassay[13] with a rat luteinizing hormone preparation (NIAMDD-RP-1) used as standard.

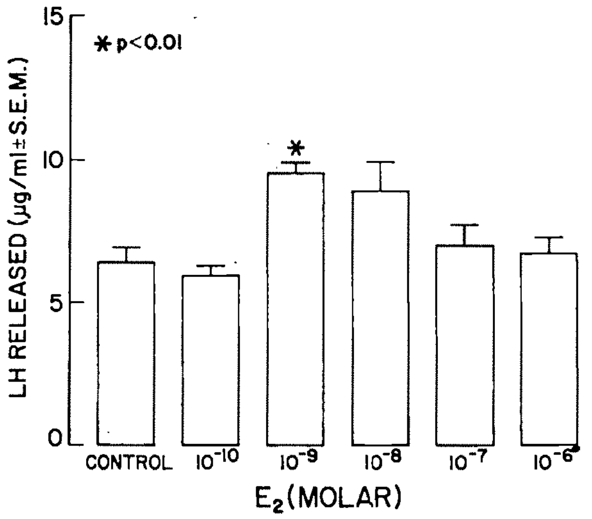

Fig. 1. Effect of increasing concentrations of estradiol on the in vitro responsiveness of pituitary cells to GnRH. Triplicate cultures were exposed to 10^{-10} to 10^{-6} mol/L of estradiol for 48 hours. Control cultures were exposed to diluent, 0.5% ethanol. At the end of this period, the medium was replaced with serum-free medium containing 10^{-8} mol/L of GnRH and incubation continued for 4 hours. At the end of the incubation period, luteinizing hormone was assayed in the medium by radioimmunoassay. Results are expressed as the mean of triplicate samples on quadruplicate determinations (*$p < 0.01$).

Incorporation of radiolabeled amino acid into protein. Protein synthesis was determined by adding 10 μCi of tritium-labeled L-leucine per culture and assaying the incorporation of radiolabeled amino acid into protein during the estradiol and control treatment periods (48 hours). Cycloheximide at a final concentration of 50 μmol/L was added during the final 8 hours of incubation. Media were then removed from the cultures; cells were washed five times with serum-free media and dissolved in 0.1N sodium hydroxide. Aliquots were neutralized with 0.1N hydrochloric and, after addition of 0.2 mg of bovine serum albumin to the samples, equal volumes of 20% trichloroacetic acid containing 2% phosphotungstic acid and 0.1% L-leucine were added. After 30 minutes on ice, the samples were centrifuged at 2500 × g for 20 minutes. The pellets were washed five times by dissolving in 1 ml of 1N

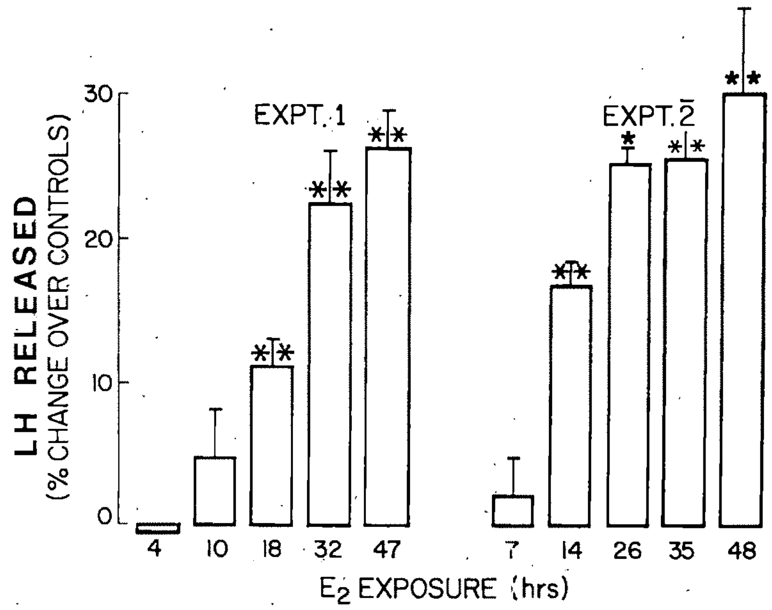

Fig. 2. Time course of the effect of estradiol exposure on the responsiveness of pituitary cells to GnRH. After cell attachment, cultures were incubated with 10^{-9} mol/L of estradiol for increasing lengths of time up to 48 hours. Controls were exposed to diluent, 0.5% ethanol. At the end of each exposure period, media were replaced with serum-free media containing 10^{-8} mol/l of GnRH and incubation continued for 4 hours. After this period, the media were separated and assayed for luteinizing hormone content by radioimmunoassay. In experiment 1, p values are calculated versus the 4-hour point; in experiment 2, p values are expressed versus the 7-hour point (**$p < 0.01$, *$p < 0.005$).

sodium hydroxide followed by precipitation with 1 ml of 1N hydrochloric acid. The final pellet was dissolved in 1 ml of NCS tissue solubilizer (Amersham Corp., Arlington Heights, Illinois) and counted in a liquid scintillation counter after the addition of 10 ml of scintillation fluid. Protein content in the dissolved cells was determined by the method of Lowry et al.[14] with bovine serum albumin used as standard.

Iodine 125–labeled D-Ala^6 GnRH-binding studies. Following the incubation with test substances, the cultured cells in each experimental group were washed, scraped free with a rubber policeman, combined, and transferred to 12 by 75 mm glass tubes at a concentration of 2 to 3 × 10^6 cells per tube for the binding studies. Radioiodinated D-Ala^6 GnRH for use as radioligand was prepared by the lactoperoxidase method.[15] The specific activity of the ^{125}I-labeled peptide was in the range of 850 to 1500 μCi/μg.

Binding assays were performed as described by Naor et al.[16] In brief, the labeled peptide (50,000 cpm) was incubated with the pituitary cells in a total volume of 0.3 ml of assay buffer (Dulbecco's modified Eagle's medium, 0.1% bovine serum albumin, 0.5 mmol/L of bacitracin, and 25 mmol/L of HEPES, pH 7.4) for 80 minutes at 22° C. Nonspecific binding was determined by including 10^{-6} mol/L of D-Ala^6 GnRH in the binding assay. Specific binding was determined by subtracting nonspecific binding from the total binding. The reaction was stopped by the addition of 2 ml of ice-cold phosphate-buffered saline (pH 7.2), followed by filtration under vacuum through Whatman GF/C filters presoaked in 1% bovine serum albumin and washing three times each with 4 ml of phosphate-buffered saline. The bound radioactivity was determined by counting the filters in a gamma spectrometer.

To study the effect of estradiol on GnRH receptor content and affinity, after estradiol exposure the cell cultures were incubated with 50,000 cpm of iodinated GnRH and increasing concentrations of unlabeled analogue from 0.25 to 2.25 nmol/L in the absence or presence of 1000-fold excess of unlabeled analogue. The amount of GnRH bound to the cells was determined at each point.

Protein determination. Protein was determined by the method of Lowry et al.[14] Cell protein content in these studies did not vary significantly in control versus estradiol-treated cultures.

Results

Initial experiments were performed to determine the effect of estradiol pretreatment on the in vitro sensitivity of the cell cultures to GnRH for luteinizing hormone release. The effect of pretreatment with different concentrations of estradiol on the subsequent ability of

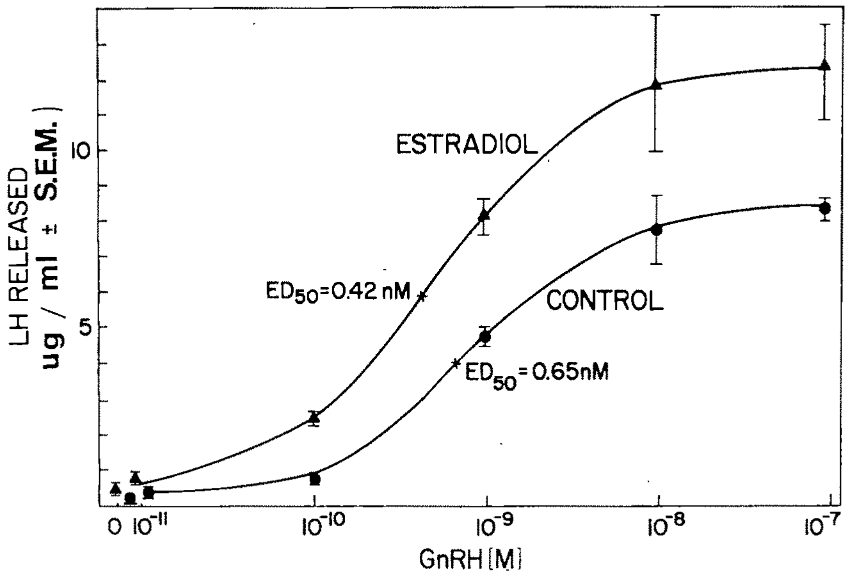

Fig. 3. Effect of increasing concentrations of GnRH on luteinizing hormone release from pituitary cells preincubated in the presence or absence of estradiol. Cultures were exposed to 10^{-9} mol/L of estradiol or control diluent for 48 hours. At the end of this time period, media were replaced with fresh serum-free media containing indicated concentrations of GnRH and incubation continued for 4 hours. Media then were separated and luteinizing hormone content measured by radioimmunoassay.

the pituitary cells to respond to exogenous GnRH for luteinizing hormone release is shown in Fig. 1. The cell cultures were treated with 10^{-10} to 10^{-6} mol/L of estradiol for 48 hours, after which the medium was replaced with serum-free medium and incubated with 10^{-8} mol/L of GnRH for 4 hours and the amount of luteinizing hormone released into the medium was determined by radioimmunoassay. It was observed that estradiol pretreatment of the pituitary cells potentiated the GnRH-stimulated luteinizing hormone release compared to that of controls; estradiol was effective at a dose of 10^{-8} mol/L, and a significant augmentation of luteinizing hormone release was obtained by exposure to 10^{-9} mol/L of estradiol. Thus in subsequent experiments a concentration of 10^{-9} mol/L of estradiol was chosen.

The time course of the effect of estradiol exposure on luteinizing hormone release in response to GnRH was then examined (Fig. 2). After incubation of the cell cultures with 10^{-9} mol/L of estradiol for 4 to 48 hours, the medium was replaced with serum-free Dulbecco's modified Eagle's medium containing 10^{-8} mol/L of GnRH and incubated for an additional 4 hours. Treatment with estradiol for a period as long as 10 hours had no effect on GnRH-stimulated luteinizing hormone secretion, whereas treatment for 14 to 48 hours increased secretion. The maximal increase in responsiveness was achieved by a 48-hour exposure time, the maximum time point tested. These results demonstrate that the duration of exposure to estradiol resulted in progressive augmentation of luteinizing hormone release in response to GnRH compared to that of controls.

The effect of estradiol on the half-maximal stimulation of GnRH was then tested. The cell cultures were exposed to 10^{-9} mol/L of estradiol or diluent for 48 hours and subsequently challenged with increasing concentrations of GnRH from 10^{-11} to 10^{-7} mol/L and the amount of luteinizing hormone released into the medium was assayed. As shown in Fig. 3, cell cultures pretreated with estradiol exhibited a marked increase in luteinizing hormone secretion compared to that of controls in response to GnRH in the concentration range tested, with maximal luteinizing hormone production significantly enhanced. The concentration of GnRH required to elicit half-maximal stimulation of luteinizing hormone release was found to be 0.42 nmol/L in the presence of estradiol whereas for control cultures it was 0.65 nmol/L.

Effect of estradiol on the incorporation of tritium-labeled L-leucine into proteins. Since estradiol has been shown to increase protein synthesis in the target cells, the possibility that the stimulatory effect of estradiol in the cultured pituitary cell also may occur by this process was examined. Results presented in Table I show that the addition of estradiol caused a significant increase in the incorporation of tritium-labeled L-leucine into trichloroacetic acid–precipitable proteins in the pituitary compared to that in controls. As expected, cycloheximide, a known inhibitor of protein synthesis, inhibited the incorporation, suggesting that the ob-

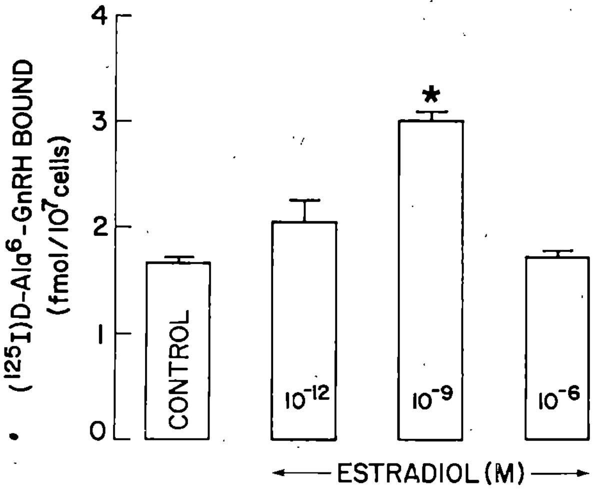

Fig. 4. Effect of estradiol on GnRH receptor content. Pituitary cells incubated for 48 hours with increasing concentrations of estradiol or control diluent were removed from culture dishes and the binding of ^{125}I-labeled D-Ala^6 GnRH was determined. Binding assay conditions were as described under Material and methods (*$p < 0.01$ versus control).

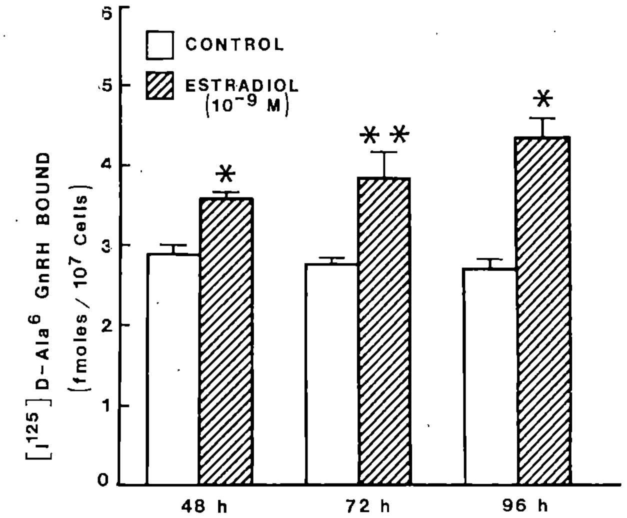

Fig. 5. Effect of estradiol exposure on GnRH binding activity. Pituitary cell cultures were incubated with diluent or 10^{-9} mol/L of estradiol for increasing lengths of time as indicated. At the end of each exposure period, cells were collected and GnRH binding studies were performed as described in Material and methods (*$p < 0.005$, **$p < 0.010$ versus controls at the same time point).

Table I. In vitro effect of estradiol on amino acid incorporation into protein

	Tritium-labeled Leucine incorporation per culture (pmol/ 100 μg protein, ± SEM)	
Addition	*Control*	*Estradiol (10^{-9} mol/L)*
None	2.7 ± 0.15	3.64 ± 0.36
Cycloheximide (50 μmol/L)	1.10 ± 0.08	1.43 ± 0.06*

After attachment, pituitary cell cultures were incubated with estradiol or control diluent (0.5% ethanol) for 48 hours in the presence of 10 μCi of tritium-labeled L-leucine. Cycloheximide was added during the final 8 hours of incubation. Samples were then processed for the determination of radiolabeled amino acid incorporation into proteins as described under Material and methods. Values are means ± SEM of triplicate determinations.

*$p < 0.005$ versus estradiol-treated cultures.

served effect is due to increased protein synthesis rather than increased precursor uptake.

Effect of estradiol pretreatment on GnRH receptor. To test whether the potentiating effect of estradiol results from an increase in GnRH receptor, binding studies were performed with pituitary cells previously cultured in the presence and absence of estradiol. Cell cultures exposed to increasing concentrations of estradiol (10^{-12} to 10^{-6} mol/L) for 48 hours exhibited a significant increase in GnRH binding capacity in those cultures exposed to 10^{-9} mol/L of estradiol compared to that of controls (Fig. 4). The effect of increasing incubation time with estradiol on GnRH binding activity at different time points is shown in Fig. 5. At each time point tested, GnRH binding activity showed a significant increase compared to that of controls; at 96 hours the difference between controls and estradiol-treated groups was maximum. To determine whether the effect of estradiol on GnRH receptors was due to a change in the affinity of or an increase in the number of GnRH binding sites, control and estradiol-treated cell cultures were assayed for GnRH binding activity with increasing concentrations of GnRH, and these data were transformed according to Scatchard[17] to determine the equilibrium dissociation constants and the number of binding sites (Fig. 6). Estradiol treatment of pituitary cells did not alter the dissociation constants, 4.94×10^{-10} mol/L, compared to control value of 4.78×10^{-10} mol/L, indicating that no change in GnRH binding affinity had occurred. Rather these results indicate that the observed increase in GnRH binding activity was due to an increase in the number of binding sites, from 4065 (control) to 10,570 per cell (Table II). This increase in receptors was prevented by the protein synthesis inhibitor cycloheximide. The inclusion of cycloheximide during a 48-hour incubation with estradiol produced a decrease in the GnRH binding capacity compared to that seen in cultures incubated with estradiol alone, suggesting that the receptor induction involves events associated with protein synthesis (Fig. 7).

Comment

The direct effect of estradiol on pituitary responsiveness has been shown to be stimulatory and inhibitory, depending on the duration and concentration of

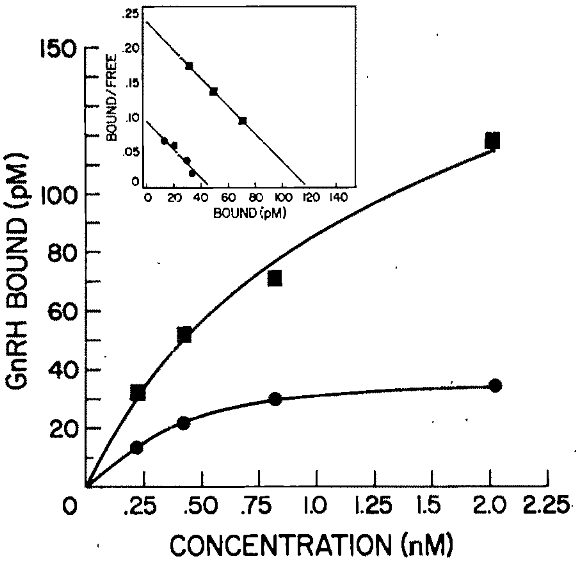

Fig. 6. Saturation curve of D-Ala^6 GnRH binding to pituitary cells maintained for 4 days in culture in the presence or absence of estradiol. Cultures were incubated with or without estradiol (10^{-9} mol/L for 48 hours, removed from culture dishes, and binding of ^{125}I-labeled D-Ala^6 GnRH was determined by use of a fixed quantity of radioligand with increasing concentrations of unlabeled analogue. Other experimental conditions were the same as described under Material and methods. Specific binding is shown on the abscissa. A Scatchard plot of these data is shown in the *inset*. The equilibrium dissociation constants are 4.8×10^{-10} mol/L and 5.0×10^{-10} mol/L for control and estradiol-treated cultures, respectively. ●——● = Control; ■——■ = estradiol (10^{-9} mol/L).

estradiol under in vivo[18, 19] and in vitro[8, 20-22] conditions. In the present experiments we have attempted to study the augmentary effect of estradiol on luteinizing hormone release. Studies by Savoy-Moore et al.[10] indicate that between metestrous and proestrous periods there is a significant rise in pituitary GnRH receptor content that is closely associated with the rise in serum estrogen levels. Tang et al.,[23] using membranes obtained from adult rat pituitaries at random stages of the estrous cycle have shown that an estradiol priming effect on pituitary luteinizing hormone response is associated with an increase in cellular luteinizing hormone content followed by an increase in GnRH receptor content. In our present study, we have used intact cultured cells from immature female pituitaries for the binding study rather than membranes from adult rats. Our studies have clearly shown that the effect of estradiol on luteinizing hormone release in response to synthetic GnRH is dependent on the concentration and duration of exposure to estradiol. The maximum augmentary response was seen at 10^{-9} mol/L of estradiol and at 48 hours of exposure time. Binding studies have shown that estradiol pretreatment causes a significant increase in the number of GnRH binding sites in rat anterior pituitary cells without any change in the affinity. These in vitro studies suggest that estradiol did increase the number of pituitary receptors for GnRH and that the potentiating effect of estradiol on luteinizing hormone release at the pituitary level might be mediated at least in part by an increase in the number of GnRH receptor sites in the pituitary. The present finding that estradiol increases GnRH receptors in pituitary cell cultures does not appear to be due to an increase in cell number, since estradiol treatment did not result in a significant increase in protein content. Although estradiol has been known to cause cell proliferation, it has not been shown, among the various cell types found in the pituitary, that proliferation of luteinizing hormone–producing cells actually takes place. Furthermore, Tang et al,[23] have reported that the increased tritium-labeled

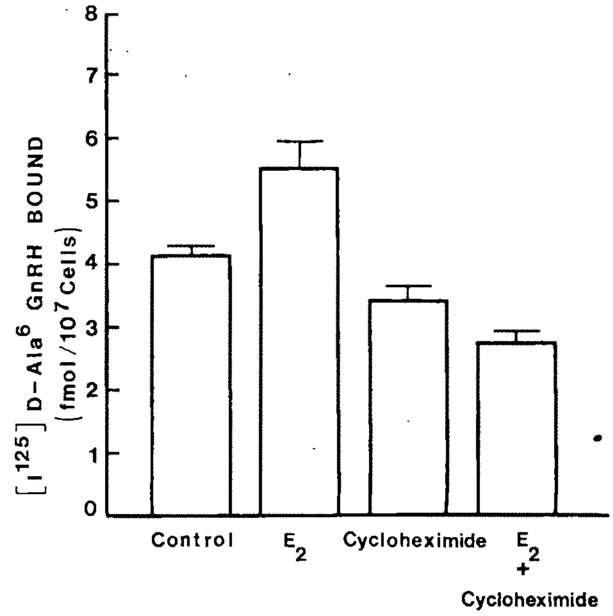

Fig. 7. Effect of cycloheximide on GnRH binding capacity. Control and estradiol (10^{-9} mol/L)–exposed pituitary cells were incubated for 48 hours in the presence and absence of 20 μmol/L of cycloheximide. After cell collection, binding of the radiolabeled GnRH was determined as described under Material and methods.

Table II. Effect of estradiol pretreatment on the binding constants of pituitary GnRH receptors

Culture	*Equilibrium dissociation constant*	*GnRH binding sites (No.)*
Control	4.78×10^{-10} mol/L	4,065
Estradiol (10^{-9} mol/L)	4.94×10^{-10} mol/L	10,570

Pituitary cells were cultured with or without estradiol for 48 hours, after which binding of the radioligand was examined by adding increasing concentrations of unlabeled analogue. Other experimental conditions were as described under Material and methods. Data taken from Fig. 6.

thymidine incorporation they observed in pituitary cells cultured in the presence of estradiol occurred considerably later than the estradiol priming effect on pituitary GnRH responsiveness.

The mechanism by which estradiol produces an effect on GnRH receptors is not understood at the present time. Current theories on protein hormone-receptor processing suggest that the receptors are internalized and processed intracellularly, resulting in the recycling of the receptors.[24] Whether estradiol causes a synthesis of new GnRH receptors or exposition of cryptic receptors by translocation from intracellular sites is not known. However, stimulation of amino acid incorporation into protein by estradiol pretreatment suggests the role of protein synthesis. It is conceivable that estradiol might cause an increase of GnRH receptors by increasing the synthesis of new protein. The observed effect is not due to a change in the number of cells, since the augmentary effect was still observed when results were expressed on the basis of protein content. The duration of estradiol exposure needed for a significant stimulation of GnRH receptors would further support the role of protein synthesis. In addition, protein synthesis inhibitors such as cycloheximide inhibited the estradiol effect on GnRH receptors by either preventing receptor synthesis or recycling the receptors to the cell surface.

In conclusion, the current studies show that estradiol augmentation of GnRH responsiveness by the pituitary is related to an increase in GnRH receptors, and the induction of receptors involves protein synthesis in the pituitary.

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The significance of C-reactive protein levels in women with premature rupture of membranes

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In a prospective study of 100 patients with preterm premature rupture of membranes, clinical chorioamnionitis was present in 18 and histologic chorioamnionitis was present in 63. Patients who were managed conservatively for premature rupture of membranes were monitored by C-reactive protein determination, white blood cell and differential counts, maternal temperature, and fetal heart tone. C-reactive protein was measured nephelometrically (Immuno-chemistry Analyzer II, Beckman). Elevated C-reactive protein levels correlated well with both the pathologic and the clinical diagnosis of chorioamnionitis. Elevated C-reactive protein levels (at least 12 to 24 hours before delivery) were more sensitive than other standard laboratory or clinical tests in predicting chorioamnionitis both by clinical and pathologic criteria. When C-reactive protein values were normal, clinical chorioamnionitis was rarely found, whereas pathologically diagnosed chorioamnionitis was found half of the time. We conclude that although the C-reactive protein level is a very sensitive predictor of infectious morbidity in premature rupture of membranes, its specificity is not high. (AM J OBSTET GYNECOL 1985;151:541-4.)

Key words: C-reactive protein, premature rupture of membranes, chorioamnionitis

The purpose of this study was to find a marker for the diagnosis of early infection in patients with premature rupture of membranes. C-reactive protein measurement had been used previously at our institution in a preliminary study for the management of patients with premature rupture of membranes as well as those with gynecologic infections. This study was planned to evaluate prospectively the sensitivity and specificity of such a test in the management of patients with premature rupture of membranes.

Material and methods

All patients admitted to Chicago Lying-in Hospital between August 1, 1980, and July 30, 1982, with premature rupture of membranes (documented by the presence of gross pooling of amniotic fluid or of Nitrazine-positive fluid in the vaginal vault), who were of <35 weeks' gestation and who had no signs or symptoms of chorioamnionitis and no labor contractions were included in this study. These patients were managed conservatively and were evaluated with the following tests: (1) A uterine cervical culture was tested for group B streptococci, *Neisseria gonorrhoeae,* and *Chlamydia trachomatis.* (2) A real-time sonogram was made to rule out gross congenital malformation and to identify pockets of amniotic fluid. (3) An amniocentesis was performed with patient consent. The fluid obtained was evaluated for fetal lung maturity (by lecithin/sphingomyelin ratio and the presence of phosphatidylglycerol) and tested for infection (by Gram stain and aerobic and anaerobic bacterial cultures). (4) Blood was drawn for a complete blood cell count with differential white cell count and for C-reactive protein determination.

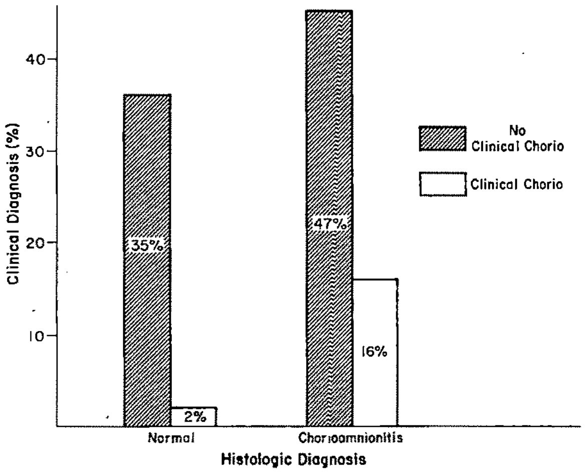

Fig. 1. Correlation between histologic and clinical chorioamnionitis.

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Table I. Demographic data of patients entered in the study

	Mean	*SEM*	*Range*	*Median*
Age (yr)	24.5	±5.2	15-39	24.6
Gestational age (wk)	31		26-37	31.8
Duration of premature rupture of membranes (hr)	150	±21.7	5-1053	72.0
Gravidity	3	±2		
Parity	2	±1		

Table II. Patterns of labor, mode and route of delivery, fetal sex, and outcome in patients of the study

Occurrence	%
Spontaneous labor	88
Induced labor	12
Spontaneous vaginal delivery	47
Outlet forceps delivery	40
Cesarean section	13
Male infant	64
Female infant	36
Live birth	98
Stillbirth*	2

*One infant weighed 535 gm and was very immature, and the second infant had a traumatic breech delivery.

rate nephelometric assay was performed to measure C-reactive protein with the use of a Beckman immunochemistry analyzer (automated model) with the C-reactive protein reagent kit (Beckman instruments, Inc., Fullerton, California). C-reactive protein results were not available for clinical management.

Following admission, daily complete blood cell and differential counts and C-reactive protein determinations were performed. Fetal heart rate, maternal temperature, and uterine tenderness or contractions were evaluated every 8 hours. Conservative management was interrupted if labor occurred or if clinical evidence of chorioamnionitis developed. Labor was induced if maternal temperature rose to 38° C or higher, if the uterus became tender and irritable, or if foul-smelling amniotic fluid was noted, and if an abnormal fetal heart rate developed, e.g., fetal tachycardia (>180 bpm). Uterine irritability alone was not an indication for delivery.

All newborn infants underwent a routine workup for sepsis and were treated with antibiotics for 3 days prophylactically. Further evaluation and management of any other neonatal problems were done in a routine manner. All placentas and amniotic membranes were histologically evaluated for evidence of inflammation and/or infection. The criteria that were used to establish a diagnosis of histologic chorioamnionitis were (1) polymorphonuclear leukocyte infiltration of the extraplacental membranes, (2) an accumulation of polymorphs in the intervillous space immediately below the chorionic plate, (3) leukocyte infiltration of the chorionic plate, and (4) angiitis of umbilical vessels.

Results

One hundred patients between 26 and 35 weeks' gestation with documented premature rupture of membranes were enrolled in the study. Demographic data are shown in Table I. The mean gestational age of these patients was 31 weeks, and the mean duration of premature rupture of membranes at the time of delivery was 150 hours; 88% went spontaneously into labor, whereas labor was induced for 12%. The cesarean section rate was 13%, as shown in Table II. Amniocentesis was attempted in 51 patients and was successful 45% of the time.

Eighteen patients developed clinical chorioamnionitis, while histologic chorioamnionitis was diagnosed in the placentas of 63 patients. Fig. 1 shows the correlation between histologic and clinical chorioamnionitis. Only 16 patients had both clinical and histologic chorioamnionitis. Of the 37 patients with no findings of histologic chorioamnionitis, only two had signs and symptoms of clinical chorioamnionitis. Of these two, one had a urinary tract infection (*Escherichia coli* culture, $>10^5$) while the other had no other source of infection.

Table III shows a comparison of the sensitivity of different tests in identifying clinical chorioamnionitis. C-reactive protein determination was the most sensitive test (82%), followed by presence of fever (56.1%) and white blood cell count (47%); fetal tachycardia was the least sensitive indicator (22%). On the other hand, when the specificities of these tests were considered, C-reactive protein was found to be the least specific.

Table IV shows a comparison of the different test capabilities in the identification of pathologic chorioamnionitis. Determination of C-reactive protein was far superior in sensitivity to all of the other tests. Its specificity was also high (81.8%) but less than that of the other tests.

Comment

A conservative approach to the management of patients with premature rupture of membranes has been advocated by several authors.[1,2] The end point of such management is either the occurrence of clinical chorioamnionitis or the onset of spontaneous labor.

Identification of patients at risk for the development of amnionitis is a difficult problem. Bobbitt and Ledger[3] have shown that the examination of amniotic fluid alone is not very reliable, since the presence or absence

Table III. Comparison of C-reactive protein determination and other tests in the identification of clinically diagnosed chorioamnionitis

Test	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
C-reactive protein*	82	55	36	91
White blood cell count†	47	85	40	89
Differential white cell count‡	33	94	55	87
Maternal temperature	56	98	83	91
Fetal heart tone§	22	97	67	87

*Values > 2 mg/dl.
†Increased by 30% ante partum over baseline values.
‡More than six bands or any toxic granulation in white cells.
§Persistent fetal heart tones > 160/min.

Table IV. Comparison of C-reactive protein determination and other tests in the identification of pathologically diagnosed chorioamnionitis

Test	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
C-reative protein*	67	81	90	50
White blood cell count†	23	86	75	40
Differential white cell count‡	14	95	90	40
Maternal temperature	17	97	90	41
Fetal heart tone§	8	97	83	38

*Values > 2 mg/dl.
†Increased by 30% ante partum over baseline values.
‡More than six bands or any toxic granulation in white cells.
§Persistent fetal heart tone > 160/minute.

of white blood cells and/or bacteria correlates poorly with the development of infection. Other investigators[4] have reported on the bacteriostatic activity of the amniotic fluid and its ability to combat infection and thus decrease the risk of amnionitis.

The use of amniocentesis in patients with premature rupture of membranes has been advocated by Garite et al.[5] Gravett et al.[6] reported on the use of gas-liquid chromatography for rapid diagnosis of amniotic fluid infection. The search continues for a reliable laboratory test to serve as an early indicator of impending infectious morbidity in patients with premature rupture of membranes. The tests used routinely to follow patients managed conservatively lack both sensitivity and specificity.

In comparing C-reactive protein levels with other laboratory tests as indicators of infection (e.g., white blood cell count, differential count, maternal temperature, fetal heart rate), we found C-reactive protein level to be more sensitive but less specific in identifying clinical chorioamnionitis. C-reactive protein is an acute phase protein[7] synthesized by hepatocytes through induction by humoral mediator—probably interleukin-1. Once released, C-reactive protein becomes bound to altered or necrotized membrane structures. Its biologic effects[8] include enhancement of phagocytosis, stimulation of leukocyte motility, and opsonic effects, suggesting a specific role in tissue regeneration and repair. The correlation with histologic chorioamnionitis was much higher for C-reactive protein determination than for the other tests with respect to sensitivity, but specificity (as noted earlier), although high for C-reactive protein (81%), was lower than that seen with the other tests. It seems clear that histologic changes precede the clinical manifestations of chorioamnionitis, and in this case it is logical to assume that C-reactive protein is an early predictor of an inflammatory process. Our results are in agreement with previous work from our institution,[9] as well as with the findings of Hawrylyshyn et al.,[10] but differ from those of Farb et al.[11] who found C-reactive protein level of no value as a pathognomonic test.

The histologic presence of chorioamnionitis in 63% of the placentas and amniotic membranes in the present study sheds further light on the incidence of subclinical infection in these patients, and it suggests that this may be a contributing factor in the etiology of premature rupture of membranes. This finding is consistent with other reports in the literature concerning this clinical condition.[12]

In our institution all placentas and amniotic membranes are examined by a perinatal pathologist, minimizing the risk of overdiagnosis of chorioamnionitis. At the Chicago Lying-In Hospital, we do not use steroids nor do we give tocolytic agents to any of our patients with premature rupture of membranes. Currently we deliver all patients with premature rupture of membranes of ≥35 weeks' gestation and those with an estimated fetal weight of ≥2500 gm. The high incidence of histologic chorioamnionitis which was found in our study patients and the positive predictive value of elevated C-reactive protein for this condition make C-reactive protein determination a valuable tool for the conservative management of premature rupture of membranes. Whether or not these histologic, subclinical changes are the cause of premature rupture of membranes remains controversial.

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Modulation of thymosin β_4 by estrogen

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The endocrine thymus produces several hormone-like peptides (generically termed thymosins) which control development of the thymic-dependent lymphoid system and participate in the process of immune regulation. In addition, recent literature supports the hypothesis that gonadal steroids in general and estrogens in particular affect the immune system. To determine whether steroid hormones modulate secretion of thymic peptides, basal concentrations of thymosins α_1 and β_4 were determined by radioimmunoassay in morning blood samples from 87 women in various clinical states. Basal concentrations of thymosin α_1 were similar in all women sampled. Basal levels of thymosin β_4 were similar in normal women during the early follicular phase, women with premature ovarian failure, postmenopausal women not receiving estrogen, and individuals with gonadal dysgenesis. However, the marked variability of basal levels in premature ovarian failure and in postmenopausal women suggests that these groups are quite heterogeneous. Thymosin β_4 concentrations were reduced in castrated women not receiving estrogen and were decreased more in both postmenopausal women and castrated women who were on chronic estrogen therapy. These data suggest that estrogens can modulate the circulating levels of thymosin β_4 but not of thymosin α_1. We do not yet know whether sex steroids modulate secretion of other thymic peptides. (AM J OBSTET GYNECOL 1985;151:544-9.)

Key words: Thymosin β_4, estrogen, thymus, peptide, radioimmunoassay

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The maintenance of immune homeostasis relies heavily on the thymus gland. Absence of the thymus results in both immunologic and endocrinologic disturbances.[1] Recently we have isolated and sequenced two thymic peptides, thymosin α_1 and thymosin β_4, with each having a different immunologic and endocrinologic activity profile. Both peptides have been localized to the epithelial cells of the thymus.[2] However, controversy still exists as to whether these peptides may also be produced in small quantities by other tissues.[3] We have also developed specific radioimmunoassays for each peptide.[4,5]

Thymosin α_1 is highly active in amplifying T cell immunity[6] and is active in modulating the expression of terminal deoxynucleotidyl transferase.[3] In addition, exogenous thymosin α_1 can increase corticosterone secretion.[7] Thymosin β_4 acts on lymphoid stem cells and may influence the early stages of the maturation process of thymus-dependent lymphocytes.[2] Also, thymosin β_4 has been shown to stimulate release of luteinizing hormone–releasing factor from superfused hypothalami in vitro[8] as well as release of luteinizing hormone following in vivo intracerebral administration.[9]

Separate evidence supports the hypothesis that gonadal steroids affect the immune system.[10] Both estrogen[11] and androgen[12] receptors have been identified in thymic epithelial cells. Immune responses are clearly altered during pregnancy when circulating gonadal steroid concentrations are extremely high,[13] and the effects of sex steroid manipulation in both spontaneous and experimentally induced autoimmune diseases are well recognized.[14-16]

In the present study we have examined circulating concentrations of thymosin α_1 and thymosin β_4 in 87 women in various clinical situations in which circulating estrogen levels differ markedly.

Material and methods

Blood samples. Basal fasting serum samples for measurement of thymosin α_1 and thymosin β_4 were collected from the following groups of men and women.

Daily samples were collected from three men for 5 days to assess thymic peptide variability from day to day. Single samples were collected from 47 women aged 19 to 64 years to determine whether any systemic changes in thymic peptides could be discerned with age. Single samples were collected from an additional 87 women representing a variety of clinical states: (1) Ten normal women in the early follicular phase of their menstrual cycles (EF). (2) Twenty women under age 35 with premature ovarian failure as previously defined by us.[17] (3) Ten postmenopausal women receiving and 14 not receiving exogenous estrogen for at least 3 months. (All subjects receiving estrogen were administered the equivalent of at least 0.625 mg of conjugated estrogen orally for the first 25 days of each month and 10 mg of medroxyprogesterone acetate orally days 16 to 25 of each month. Basal blood samples were obtained between days 10 and 15 of the month while subjects were receiving estrogen alone.) (4) Fourteen castrated women receiving and 14 not receiving exogenous estrogen for at least 3 months. (These women were oophorectomized at least 1 year prior to study.) (5) Five women with gonadal dysgenesis.

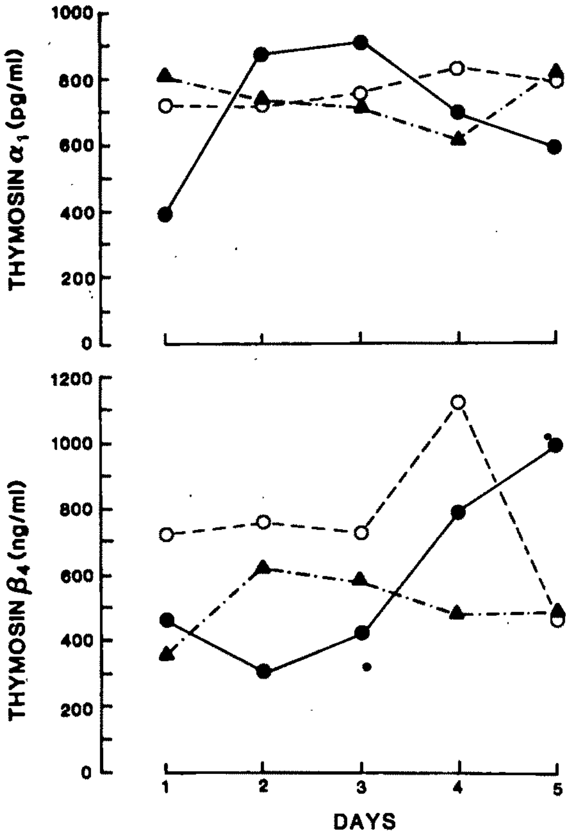

Fig. 1. Daily concentrations of circulating thymosin α_1 and thymosin β_4 in three men. Marked variability is apparent from day to day.

In addition, samples were collected at 2- to 3-day intervals from four women through a single menstrual cycle. Samples also were collected at frequent intervals from four agonadal women from 2 hours before to 4 hours following a 4-hour intravenous infusion of estradiol-17β at 50 mcg/hr.

Sera from the samples were stored at −20° C until assayed for thymosin α_1 and thymosin β_4 by radioimmunoassay. For each thymic peptide all samples were measured in the same assay.

Radioimmunoassay. Thymosin α_1 was measured by radioimmunoassay as described by McClure et al.[4] The assay is capable of detecting as little as 100 pg/ml of

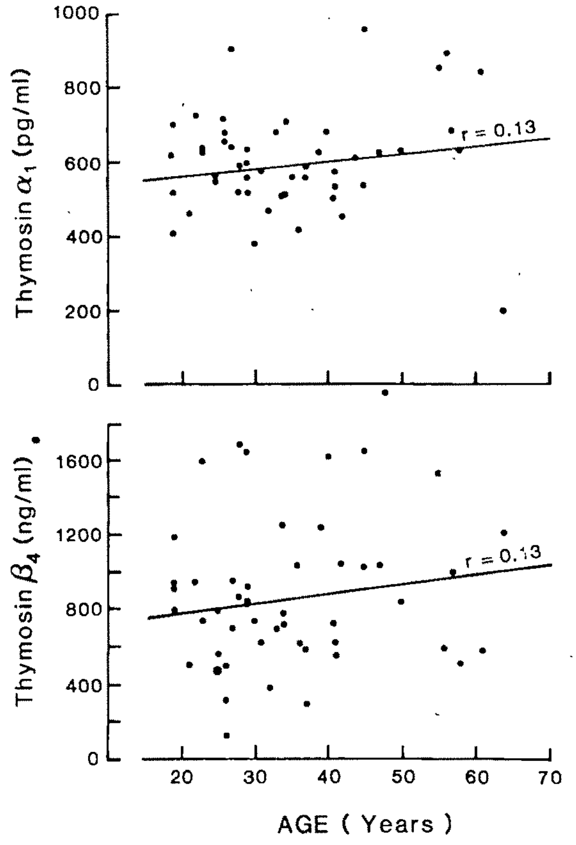

Fig. 2. Serum concentrations of thymosin α_1 and thymosin β_4 in random samples obtained from 47 men and women at the time of blood donation. No information regarding the stage of the menstrual cycle or the ovarian status of the women was available. More variability is apparent in thymosin β_4 than in thymosin α_1 levels. No significant changes in levels occurred with increasing age ($r = 0.13$).

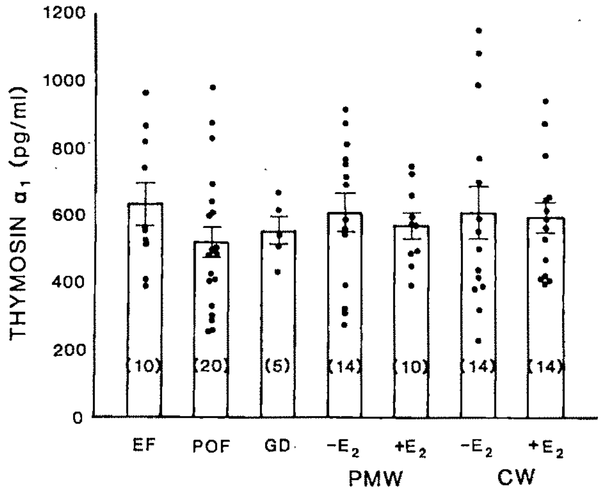

Fig. 3. Mean (± SE) concentrations of thymosin α_1 in samples obtained from 87 women representing a variety of clinical states. The values obtained for the individual subjects are depicted by the points and show marked variability. Levels of thymosin α_1 were similar in all groups of women. *EF* designates women in the early follicular phase of the menstrual cycle; *POF*, women with premature ovarian failure; *GD*, individuals with gonadal dysgenesis; *PMW*, postmenopausal women; *CW*, castrated women; $-E_2$, without estrogen therapy; $+E_2$, with estrogen therapy.

thymosin α_1 in serum or plasma. The within-assay coefficient of variation was ≤10%. Thymosin β_4 was measured by radioimmunoassay as described by Naylor et al.[5] The assay is capable of detecting as little as 5 ng/ml of thymosin β_4 in serum or plasma. The within-assay coefficient of variation was 15%.

Statistics. One-way analysis of variance and Duncan's multiple range test were used for statistical analysis.

Results

Serum concentrations of thymosin α_1 and thymosin β_4 can fluctuate considerably from day to day in the same men (Fig. 1). In individual men and women of various ages from whom samples were obtained at the time of blood donation, thymosin β_4 levels ranged from approximately 150 to 1600 ng/ml, and thymosin α_1 levels ranged from about 200 to 950 pg/ml (Fig. 2).

Levels of thymosin α_1 were similar in all of the groups of women examined and averaged 581.0 pg/ml (range, 23[illegible] to 1148 pg/ml) (Fig. 3).

Basal levels of thymosin β_4 were similar in women in the early follicular phase (487.8 ng/ml ± 113.9 SEM), patients with premature ovarian failure (529 ± 114.7), postmenopausal women not receiving estrogen (382.4 ± 154.0), and subjects with gonadal dysgenesis (697 ± 257.2) (Fig. 4). However, the marked variability of basal levels in premature ovarian failure (range, 40 to 1800 ng/ml) and in menopausal women (range, 15 to 1832 ng/ml) suggests that these groups are quite heterogeneous. Concentrations of thymosin β_4 were lowered in castrated women (191.2 ± 29.9) and were significantly reduced in both postmenopausal women and castrated women receiving chronic estrogen therapy (40.6 ± 12.3 and 63.7 ± 13.1 ng/ml; $p < 0.01$ for each comparison) (Fig. 4).

In addition, no consistent changes in either thymic peptide were observed in four normal women sampled at various times during their menstrual cycles (Figs. 5 and 6) and in four agonadal women during and immediately following a 4-hour infusion of estradiol-17β (50 mcg/hr intravenously) (Figs. 7 and 8).

Comment

These measurements indicate that there can be variability in circulating levels of both thymosin α_1 and thymosin β_4 among individuals from day to day. Levels of thymosin β_4 were more variable than those of thymosin α_1. Although mean concentratioins of thymosin α_1 were similar for all groups examined, there were differences in the mean levels of thymosin β_4, suggesting a mod-

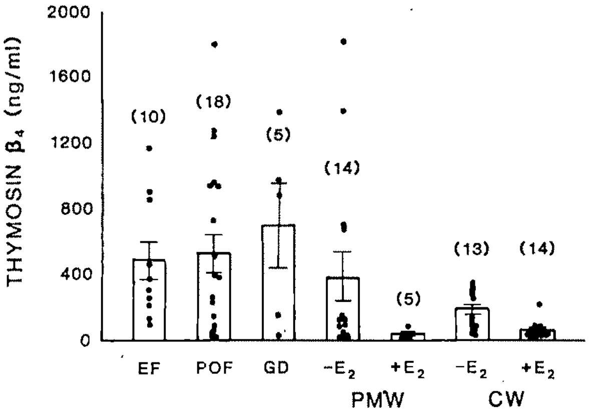

Fig. 4. Mean (± SE) concentrations of thymosin β_4 in samples obtained from 87 women representing a variety of clinical states. The values obtained for the individual subjects are depicted by the points and show marked variability. Levels of thymosin β_4 were similar in women in early follicular phase *(EF)*, women with premature ovarian failure *(POF)*, postmenopausal women *(PMW)* not receiving estrogen, and individuals with gonadal dysgenesis *(GD)*. However, the variability in *POF* and in *PMW* suggests these groups are heterogeneous. Concentrations of thymosin β_4 were significantly reduced in castrated women *(CW)* and were lower still in both postmenopausal and castrated women receiving chronic estrogen therapy.

ulating influence of gonadal steroids, specifically estrogen. Since the reduced levels of thymosin β_4 observed in agonadal women decreased further with long-term estrogen therapy, the nature of this influence remains to be defined. However, it appears that long-term estrogen therapy may lead to an adjustment in circulating concentrations of thymosin β_4. Why acute estrogen had no effect on thymosin β_4 is unclear. In addition, there was no correlation between the levels of estradiol measured by radioimmunoassay in the agonadal women and the concentrations of thymosin β_4 or thymosin α_1 (data not shown).

These findings are in agreement with our earlier data showing that thymosin β_4 but not thymosin α_1 stimulates release of luteinizing hormone–releasing factor from superfused hypothalami in vitro.[8] Thus there is good evidence that thymosin β_4 plays some role in reproductive function. These observations may also help explain the presence of estrogen[11] and androgen[12] receptors in thymic epithelial cells. They suggest the existence of a thymic-hypothalamic-pituitary-gonadal axis.

It is now clear that the endocrine thymus produces a group of hormone-like peptides which controls development of the thymic-dependent lymphoid system and participates in the process of immune regulation.[2] Our data suggest that thymic peptides also interact with the hypothalamic-pituitary unit.

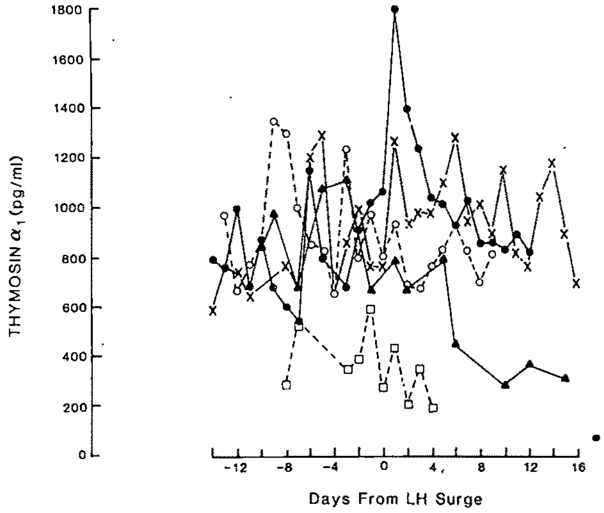

Fig. 5. Concentrations of thymosin α_1 in four women throughout a menstrual cycle. The data are centered about the day of the luteinizing hormone surge as determined by radioimmunoassay. Marked variability from day to day and among subjects is obvious. There are no systematic changes throughout the menstrual cycle.

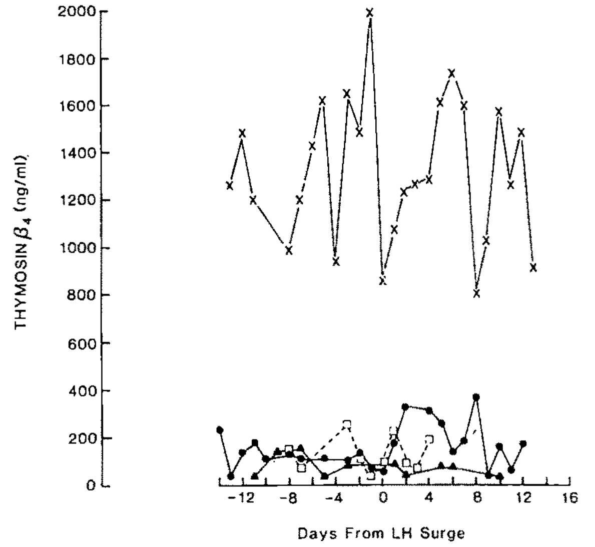

Fig. 6. Concentrations of thymosin β_4 in four women throughout a menstrual cycle. The data are centered about the day of the luteinizing hormone surge as determined by radioimmunoassay. Marked variability from day to day and among subjects is obvious. There are no systematic changes throughout the menstrual cycle. The one subject with elevated levels had variable and elevated levels throughout the cycle.

Although Calzolari[18] first suggested a relationship between the thymus gland and reproductive function in 1898, few experiments were conducted in this area until the last several years. Nishizuka and Sakakura[19] then demonstrated that neonatal thymectomy in the mouse within the first 72 hours of life leads to ovarian dysgenesis, which is first apparent at 2 to 3 months and which occurs independently of wasting disease. A

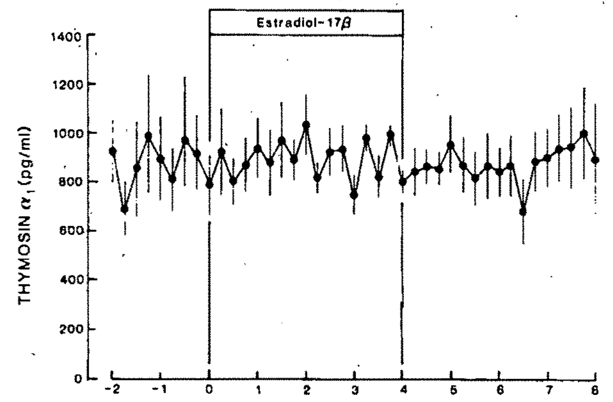

Fig. 7. Mean (±SE) serum concentrations of thymosin α_1 in four agonadal women before, during, and following infusion of estradiol-17β (50 μg/hr). No significant changes were noted.

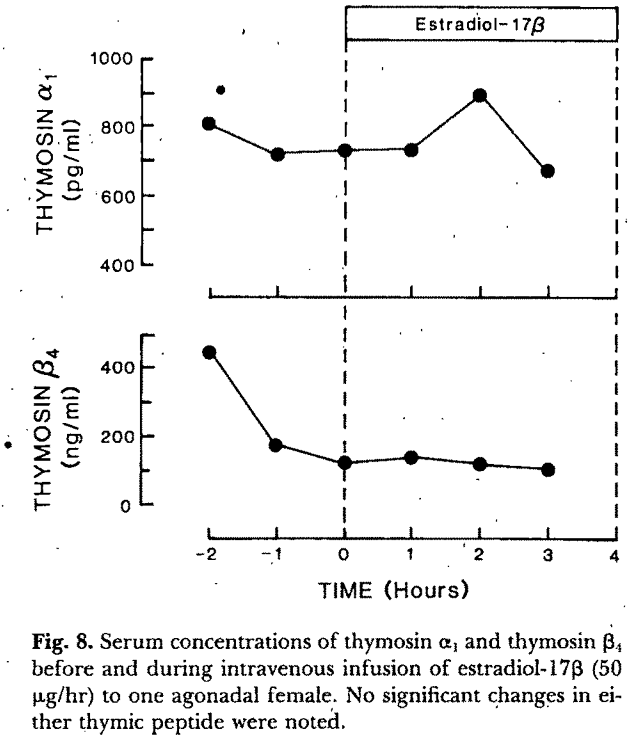

Fig. 8. Serum concentrations of thymosin α_1 and thymosin β_4 before and during intravenous infusion of estradiol-17β (50 μg/hr) to one agonadal female. No significant changes in either thymic peptide were noted.

about the same time Flanagan[20] reported breeding a new strain of congenitally athymic nude mice in which females had decreased fertility. It has since been established that affected females also have delayed vaginal opening[1] and accelerated follicular atresia such that the mice develop premature ovarian failure.[21, 22]

We reported decreased levels of luteinizing hormone and follicle-stimulating hormone in the pituitary gland and in the circulation prior to vaginal opening (i.e., puberty) and decreased circulating estrogen levels in adult athymic mice.[23] In addition, we observed reduced concentrations of immunoreactive luteinizing hormone–releasing factor in the hypothalami of athymic mice relative to normal mice at 10, 20, and 30 days of age.[24] Furthermore, the abnormalities in gonadotropin levels can be prevented by thymic transplantation in athymic nude mice at birth.[25] These findings led us to postulate that the thymus gland might exert its effects on reproduction through a humoral factor that influences the hypothalamic-pituitary unit. Furthermore, these postulates led to our subsequent studies[8] demonstrating that thymosin β_4 is capable of stimulating secretion of luteinizing hormone–releasing factor from superfused hypothalami in vitro.

The data we report here are consistent with interaction of thymosin β_4 and the hypothalamic-pituitary-gonadal system in humans. Other data are suggestive of such a relationship as well. It has long been known that young girls with congenital absence of the thymus gland have no oocytes in their ovaries at autopsy.[26] Thus thymic peptides may be important in utero in preventing accelerated follicular atresia by stimulating luteinizing hormone–releasing factor/gonadotropin secretion. Perhaps, as Ross and Vande Wiele[27] have recently suggested, gonadotropins may be necessary for maintenance of fetal oocytes.

Thymic peptides may even play a role in the activation of luteinizing hormone–releasing factor/luteinizing hormone secretion at puberty. In the human the size of the thymus gland peaks just prior to puberty,[28] simultaneous with activation of the hypothalamic-pituitary axis. Michael et al.[29] have reported that thymosin fraction 5 accelerated vaginal opening when administered to pepubertal mice. McGillis et al.[7] have recently reported that administration of thymosin α_1 results in increased corticosterone secretion in mice. Most recently, Healy et al.[30] have reported that intravenous administration of thymosin fraction 5 to prepubertal cynomolgus monkeys elevated peripheral levels of corticotropin, β-endorphin, and cortisol in a dose- and time-dependent fashion. Therefore, one might hypothesize that thymic peptides play a role in adrenarche in humans as well.

The roles thymic peptides play in the regulation of luteinizing hormone–releasing factor/luteinizing hormone secretion and the regulation of other hypothalamic factors remain unknown in humans. Further research into these areas is necessary to validate these hypotheses. Our data point to the need for continuing studies in this area.

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Dr. J. Benjamin Younger, M.D.
Professor and Director
Division of Endocrinology and Infertility
Department of Obstetrics and Gynecology
The University of Alabama at Birmingham
Birmingham, AL 35294

Deadline for applications:
April 1, 1985

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**CURRENT CONCEPTS &
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Saturday, April 13, 1985

7 Credit Hours, Category 1, A.M.A.'s Physician's Recognition Award (A.C.O.G. approval pending)

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TRIPHASIL*—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's.

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Anytime patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

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